UNIFORMED SERVICES UNIVERSITY OF THE HEALTH SCIENCES
F. EDWARD HÉBERT SCHOOL OF MEDICINE
4301 JONES BRIDGE ROAD
BETHESDA, MARYLAND 20814-4799

May 21, 2007

**BIOMEDICAL GRADUATE PROGRAMS**

***Ph.D. Degrees***

Interdisciplinary
-Emerging Infectious Diseases
-Molecular & Cell Biology
-Neuroscience

Departmental
-Clinical Psychology
-Environmental Health Sciences
-Medical Psychology
-Medical Zoology
-Pathology

Doctor of Public Health (Dr.P.H.)

Physician Scientist (MD/Ph.D.)

***Master of Science Degrees***

-Molecular & Cell Biology
-Public Health

***Masters Degrees***

-Comparative Medicine
-Military Medical History
-Public Health
-Tropical Medicine & Hygiene

***Graduate Education Office***
Dr. Eleanor S. Metcalf, Associate Dean
Janet Anastasi, Program Coordinator
Tanice Acevedo, Education Technician

***Web Site***
www.usuhs.mil/geo/gradpgm_index.html

***E-mail Address***
graduateprogram@usuhs.mil

***Phone Numbers***
Commercial: 301-295-9474
Toll Free: 800-772-1747
DSN: 295-9474
FAX: 301-295-6772

APPROVAL SHEET

Title of Dissertation: "Haplotype Analysis of the Melanopsin Gene in Seasonal Affective Disorder and Controls"

Name of Candidate: Kathryn Roecklein
Doctor of Philosophy Degree
19 June 2007

Dissertation and Abstract Approved:

David Krantz, Ph.D. — 6-19-07 — Date
Department of Medical & Clinical Psychology
Committee Chairperson

Kelly Rohan, Ph.D. — 6-19-07 — Date
Department of Medical & Clinical Psychology
Committee Member

Tracy Sbrocco, Ph.D. — 6-19-07 — Date
Department of Medical & Clinical Psychology
Committee Member

Ann Scher, Ph.D., — 6.19.07 — Date
Department of Preventive Medicine & Biometrics
Committee Member

Ignacio Provencio, Ph.D. — 6-19-07 — Date
University of Virginia
Committee Member

Copyright Statement

The author hereby certifies that the use of any copyrighted material in this thesis manuscript entitled:

**"Haplotype Analysis of the Melanopsin Gene in Seasonal Affective Disorder"**

beyond brief excerpts is with permission of the copyright owner, and will save and hold harmless the Uniformed Services University of the Health Sciences from any damage which may arise from such copyright violations.

Kathryn Ariel Roecklein, M.S.

Department of Medical and Clinical Psychology

Uniformed Services University of the Health Sciences

Abstract

Title of Dissertation: Haplotype Analysis of the Melanopsin Gene in Seasonal Affective Disorder

Kathryn Ariel Roecklein, Doctor of Philosophy, 2007

Dissertation directed by: Kelly J. Rohan, Ph.D.

Assistant Professor

Department of Psychology, University of Vermont

Seasonal affective disorder (SAD) is characterized by winter depressive episodes and springtime remission. SAD may result from a genetically mediated abnormal response to low light availability during winter. One candidate gene for SAD is melanopsin, a non-visual photopigment. Variations in the gene for melanopsin may raise the threshold of light input needed for euthymic functioning such that low light levels fall below this threshold during winter in individuals with SAD. The present study investigated the haplotype structure of the melanopsin gene by genotyping loci throughout the gene as well as in up- and down-stream regions proximal to the gene. Genotyping was performed using two strategies, 5' exonuclease assays that employ strands of about 20 DNA base pairs complementary to variant sequences in the gene, and direct sequencing of certain segments of the gene. This study tested associations between both single-base variations and specific haplotypes in the melanopsin gene in SAD participants ($n$ = 132) relative to low seasonality controls ($n$ = 90) with no history of psychopathology. This study also tested associations between seasonality (i.e., degree of seasonal variation in mood and behavior) and both polymorphisms and specific haplotypes among SAD participants. Although SAD and control participants did not differ on the overall genotype or allelic distributions for the single-base variant P10L, SAD

participants had a higher frequency of the homozygous minor allele genotype (T/T; $n = 7$, 5%) than controls ($n = 0$) when compared to the combined frequency of the C/C and C/T genotypes. No other single-base variants and none of the identified haplotypes were associated with SAD diagnosis or with seasonality. These findings suggest that genes other than melanopsin in the non-visual light input pathway, circadian clock genes, or neurotransmitter genes may be more important in the genetic risk for SAD than melanopsin.

# HAPLOTYPE ANALYSIS OF THE MELANOPSIN GENE IN SEASONAL AFFECTIVE DISORDER

By

Kathryn Ariel Roecklein, M.S.

Dissertation submitted to the Faculty of the
Medical and Clinical Psychology Graduate Program
Uniformed Services University of the Health Sciences
in partial fulfillment of the requirements for the degree of
Doctor of Philosophy, 2007

## DEDICATION

This work is dedicated to the memory of Violet Phyllis Andrews Dalton, my grandmother, and the first woman in our family to achieve an advanced degree, from the University of California at Berkley in 1935.

## ACKNOWLEDGMENTS

I would like to express my sincere thanks and gratitude to my primary academic advisor and mentor, Dr. Kelly Rohan for her tireless efforts, patience, and guidance. Dr. Rohan supported my investigation of this project and collaboration with Drs. Provencio, Rollag, and Lipsky, and encouraged me to explore interdisciplinary research. Her trust in me encouraged me to tackle a project requiring a great deal of independence and initiative. Dr. Rohan has been a true mentor, helping me to succeed in every area of the dissertation, from beginning to end.

I would like to thank Dr. Ignacio Provencio and Dr. Mark Rollag for mentoring me, helping me to develop the skills necessary to perform this type of research, and supporting the work. Without their enthusiasm and guidance during this project, I would not have thought it was possible. I would like to thank Dr. Robert Lipsky for helping to educate me in genetics, and providing the support and laboratory assistance for completing much of the data collection, and especially the encouragement and guidance needed to help me understand the genetics and statistics involved. Collaboration with Drs. Provencio, Rollag, and Lipsky was a great experience for me in the successful conduct of research and the philosophy of science. Each of these individuals encouraged me, educated me, and made me think I could complete the project. I would like to extend a very special thank you to my dissertation chair, Dr. David Krantz for his support of this dissertation project and career advice encouraging me to pursue behavioral genetics. I would also like to thank Dr. Tracy Sbrocco and Dr. Ann Scher for their comments, suggestions, and encouragement in the dissertation process.

I would like to thank my fellow lab members and friends who helped with this project including: Kathryn Tierney Lindsey, Rob Lippy, Leigh Johnson, Amy Nguyen, Aisha

Massac, Tida Kumbalasiri, and Dhrit Mukherje. Their day-to-day assistance and support were invaluable and made this research possible.

I would like to thank my family and friends, without whom no part of graduate school or this dissertation would have been possible. My friends have listened to me complain how hard it is, and even gone on to their own graduate work, while being supportive, constant, and a great source of escape from the world of graduate school. My sister Elaine encouraged me and supported me through some of the most difficult times. My mother and father have helped in every way possible, from giving me the idea in the first place, through believing I could do it when it seemed impossible, and keeping me from quitting by helping me realize it would all be worth the struggle. My sister Anne helped by listening when it was hard, no matter how many times that was the case.

Table of Contents

List of Tables

List of Figures

# Introduction

A strong rationale supports the investigation of the melanopsin gene as a candidate gene for seasonal affective disorder (SAD). Winter depression or SAD is a recurrent subtype of depression with episode recurrence in the fall and winter (Rosenthal et al., 1984b). Clinical findings which include seasonal presentation and abnormal responses to light, combined with basic molecular and animal studies suggest a neurobiological component to SAD etiology (Sohn & Lam, 2005). Genetic variants may underlie a biological predisposition to SAD. However, previously identified genetic factors associated with SAD account for only a small percentage of the estimated variance in SAD diagnosis (Rosenthal et al., 1998), and more research on genetic factors associated with SAD is needed to identify remaining sources of genetic risk. Abnormal responses to light observed in SAD suggest that the non-visual light input pathway (i.e., the pathway by which light information is conveyed to non-visual areas of the brain) is disrupted in individuals with SAD. Melanospin, a non-visual photopigment, is found in retinal ganglion cells projecting to non-visual centers of the brain (Qiu et al., 2005; Rollag, Berson, & Provencio, 2003). It is possible that sequence variations in the melanopsin gene may impact non-visual light input to the brain, making certain individuals more vulnerable to developing SAD. The present study uses a candidate gene approach, focusing on the melanopsin gene because of its plausible biological role in SAD, to investigate the potential involvement in SAD of haplotypes (combinations of closely linked loci that are inherited together), in the melanopsin gene in addition to specific loci with possible causal effects.

To provide a rationale for melanopsin as a candidate gene for SAD in the present study, the review below will describe melanopsin and its function, as well as abnormal

responses to light in SAD. Previously proposed etiological theories of SAD will be described, followed by previous research on the genetics of SAD. An in-depth discussion of study design issues and methodology for testing gene-disorder associations follows to explain the rationale for the present study proposal. Methodological issues discussed include the proposed case-control study design, the use of haplotype structure in association testing, and sources of bias in case-control studies including population stratification. Given the high risk of false-positive results in case-control studies of gene-disorder associations, a detailed discussion of how the present study will minimize this risk is presented.

## Background

### *Melanopsin*

Melanopsin is a nonvisual photopigment that is likely to be involved in SAD. This study focuses on melanopsin, a non-visual photopigment recently identified by Ignacio Provencio and colleagues (Provencio, Jiang, De Grip, Hayes, & Rollag, 1998; Provencio, Rollag, & Castrucci, 2002). We propose melanopsin is a candidate gene, a gene suspected of being involved in the expression of a trait such as SAD. After light enters the retina, two pathways with distinct visual and non-visual functional consequences are evident. In one pathway, classic visual photoreceptors (i.e., rods and cones) project to areas of the brain involved in image formation for vision. The visual photoreceptors are unlikely to be involved in SAD etiology because there is no evidence that individuals with SAD have disrupted vision. In another pathway, melanopsin containing retinal ganglion cells project to brain areas responsible for non-visual functions such as circadian photoentrainment, the pupillary light reflex, and acute melatonin suppression (Panda et al., 2003).

#### *Role for Melanopsin in Non-Visual Responses to Light in Rodents*

In rodents, melanopsin is expressed in a subset of intrinsically photosensitive retinal ganglion cells (ipRGCs; Lucas et al., 2003), that project to the master circadian clock in the suprachiasmatic nucleus (SCN) and to other non-visual centers in the brain (Gooley, Lu, Fischer, & Saper, 2003; Hattar, Liao, Takao, Berson, & Yau, 2002; Morin, Blanchard, & Provencio, 2003). Melanopsin artificially expressed in non-retinal mammalian cells leads to the acquisition of photosensitivity, strongly suggesting that melanopsin acts as a photopigment in ipRGCs (Melyan, Tarttelin, Bellingham, Lucas, & Hankins, 2005; Qiu et al., 2005). Studies with rodents have shown that melanopsin is necessary, but not sufficient, for normal non-visual responses to light including the pupillary light reflex, regulation of the circadian clock, suppressing melatonin release, and suppressing physical activity (Panda et al., 2003; Panda et al., 2002; Ruby et al., 2002).

*Role for Melanopsin in Non-Visual Responses to Light in Humans*

Rodent and primate research provides support for an analogous role for melanopsin-based irradiance-detection in humans. Primates have 'giant' ipRGCs containing melanopsin that project centrally and may contribute to vision, although potential non-visual contibutions of these cells in primates have not been fully explored (Dacey et al., 2005). The gene for melanopsin in humans has been identified and characterized as an opsin found only in retinal ganglion cells, which likely project to the SCN (Provencio et al., 2000). The role of melanopsin in non-visual behavioral responses in rodents, the recent identification of the human melanopsin gene, and the likely role of light in SAD etiology, implicates melanopsin as an appropriate candidate gene for SAD.

## *Seasonal Affective Disorder*

### *Description of Seasonal Affective Disorder*

SAD is a subtype of major depression, a debilitating mental illness characterized by high rates of recurrence and relapse with a significant social cost. SAD comprises 10-20% of all cases of depression (Blazer, Kessler, & Schwartz, 1998; Magnusson, 2000), affecting approximately 5% of individuals in the U. S. (Magnusson & Boivin, 2003). Rosenthal-National Institute of Mental Health criteria for SAD have been used to diagnose SAD since description of the syndrome in 1984, and include a history of major affective disorder according to Research Diagnostic Criteria (RDC; Spitzer, Endicott, & Robins, 1978), at least two consecutive years in which depression developed in fall or winter and remitted during the following spring or summer, the absence of any other Axis I psychiatric disorder, and the absence of any clear-cut seasonally changing psychosocial variable that could account for the seasonal variation in mood and behavior (Rosenthal et al., 1984b). Rosenthal et al. (1984) and others found that SAD is most commonly characterized by atypical or reverse vegetative symptoms including fatigue, hypersomnia, carbohydrate craving, weight gain, afternoon slump in mood or energy, and reverse diurnal variation (Lam, Tam, Yatham, Shiah, & Zis, 2001; Rosenthal et al., 1984b). However, Diagnostic and Statistical Manual (DSM-III-R) criteria for SAD, those used in the present study, include that any symptom presentation meeting criteria for a pattern of major depressive episodes that recur during a particular time of year such as fall or winter, episodes are not due to seasonal-related psychosocial stressors, full remission is experienced at a particular time of year such as spring, over the last 2 years seasonal episodes have occurred and no non-seasonal episodes have occurred, and over the individual's lifetime, seasonal episodes outnumber nonseasonal episodes 3:1 (APA, 1987). Criteria for a major depressive episode remained the same from DSMIII-R to DSM-IV-TR and include either depressed mood and/or a loss of interest in activities, as well as a change

in weight or appetite, insomnia or hypersomnia, psychomotor retardation or agitation, fatigue or loss of energy, feelings of worthlessness or inappropriate guilt, difficulty thinking or concentrating, or recurrent thoughts of death or suicidal ideation for a total of at least 5 symptoms, including at least one of the first two (APA, 2000).

*Epidemiological Findings for SAD*

Epidemiological findings regarding the age of onset and prevalence of SAD over the lifespan are important for genetic studies, and are reviewed here. The average age of onset of SAD is 27.2±11.6 years (Rosen et al., 1990). The average age of onset of Major Depressive Disorder (MDD), regardless of seasonality is 30.5 years with a standard error of 0.45, as measured in the National Epidemiologic Survey on Alcoholism and Related Disorders utilizing a sample of more than 43,000 adults in the U.S. (Hasin, Goodwin, Stinson, & Grant, 2005). The rate of SAD in adolescents is estimated to be 3.3% (Swedo et al., 1995), and the rate in children 9-12 years of age is estimated to be 4.2% (Carskadon & Acebo, 1993), suggesting that SAD is less common in the young. However, it is unclear if these rates are significantly lower than the observed rate of SAD of about 5% for adults in the U. S., averaged across latitudes (Rosen et al., 1990). Rates of MDD increase rapidly between the ages of 12 and 16 (Hasin et al., 2005), and puberty is associated with a higher rate of SAD in girls but not boys (Magnusson & Partonen, 2005). These data are difficult to interpret because the overall risk of depression is higher for more recent cohorts than older cohorts, when considering cumulative probablility of lifetime depression (Kessler et al., 2003). Explanations for why SAD and MDD appear to have an average age of onset after puberty include both biological and social effects of the status and timing of puberty (Kessler, 2002), including changes in sex hormones that can account for the difference in rates of depression

between boys and girls (Angold, Costello, & Worthman, 1998), and the increased stress of dating on girls as opposed to boys at this age (Joyner & Udry, 2000).

*SAD: Abnormal Response to Light*

As reviewed below, SAD can be characterized as an abnormal response to light for the following reasons: (1) SAD prevalence, recurrence, and symptom severity correlate negatively with photoperiod and light availability and positively with latitude; (2) circadian rhythms may be dysregulated in SAD; (3) individuals with SAD may have retinal sub-sensitivity; and (4) bright light therapy alleviates depressive symptoms in SAD. These four points are used to substantiate a plausible mechanism for the involvement of the melanopsin gene in SAD and to develop a rationale for the proposed candidate gene analysis in the sections that follow. Concurrent with the discussion of abnormal light responses in SAD, etiological theories of SAD are discussed including biological theories and integrative theories that include biological, psychological, and environmental factors.

*Integrative Hypotheses for SAD*

Most research on SAD has focused on testing biological factors in SAD pathophysiology, but it is important to review theories that propose biological, psychological, and environmental factors to explain SAD because it underscores the point that biological factors, including genetic factors, are not expected to explain all of the variance in SAD. Although the present study investigates a potential genetic factor to explain SAD, this genetic factor is not expected to explain a majority of the variance in SAD, but is expected to be one of multiple genes, and one factor among other, non-genetic factors, involved in SAD etiology. Theories reviewed here include the dual vulnerability hypothesis as proposed by

Young, the revision of this hypothesis by Lam, and an Integrative, Cognitive-Behavioral Model by Rohan.

*The Dual Vulnerability Hypothesis*

The dual vulnerability hypothesis was the first to incorporate psychological and physiological components to explain the development of SAD (Young, Watel, Lahmeyer, & Eastman, 1991). According to the model, individuals with seasonal depression have two separate vulnerabilities. First, they may have a predisposition to experiencing reverse vegetative symptoms (e.g., increased sleep, increased appetite and carbohydrate craving, and increased fatigue) in response to the changing seasons, which constitutes a "physiological vulnerability." Second, in response to the physiological symptoms, cognitive and affective mechanisms are activated, which constitute the psychological component of the "dual" vulnerability. Young tested this model by retrospectively measuring the temporal onset of reverse vegetative, cognitive, and behavioral symptoms and found that fatigue, hypersomnia, and carbohydrate craving were associated with time of onset of a SAD episode, but other symptoms including cognitive and behavioral symptoms were not related to onset, suggesting two separate mechanisms for these different symptom clusters (Young et al., 1991).

*Elaboration of Dual Vulnerability Hypothesis*

Young et al.'s (1991) dual vulnerability model has been revised by Lam et al. (2001). Lam et al. proposed that there are two "loading factors" that may contribute to whether depressive symptoms are expressed in a purely seasonal, nonseasonal, or mixed pattern of recurrence (Lam et al., 2001). These two factors, the seasonality factor and the depression factor, are proposed to differ in strength across diagnoses [(i.e., SAD, subsyndromal SAD (S-SAD), and Major Depressive Disorder (MDD)]. In S-SAD, there is little to no loading on

the depression factor and primary loading on the seasonality factor. This combination is expressed through the individual's experience of the reverse vegetative symptoms with no concomitant experience of the affective and cognitive symptoms essential for a diagnosis of a Major Depressive Episode. An individual with primary loading on the depression factor and minimal loading on the seasonality factor would most likely experience nonseasonal MDD, and would experience "typical" nonseasonal depression symptoms including loss of appetite and insomnia. SAD and SAD with incomplete summer remission are represented by intermediate loadings on the depression and seasonality factors. In the case of primary loading on the seasonality factor, the most likely expression is that of diagnosable SAD and the experience of complete remission of symptoms in spring and summer. Alternatively, higher loading on the depression factor, as compared to the seasonality factor, results in expression of a winter Major Depressive Episode without full remission of symptoms coinciding with the arrival of spring and summer. Lam et al.'s (2001) revision of the dual vulnerability hypothesis does not specify the mechanisms behind the seasonality and depression vulnerabilities (i.e., physiological, environmental, or psychological). However, this model will be useful below in the discussion of genetic factors for SAD, some of which have been implicated in both SAD and MDD. Lam et al.'s (2001) dual vulnerability hypothesis provides a framework for discussing a distinction between etiological factors in SAD that are specific to SAD, versus those that are shared with MDD.

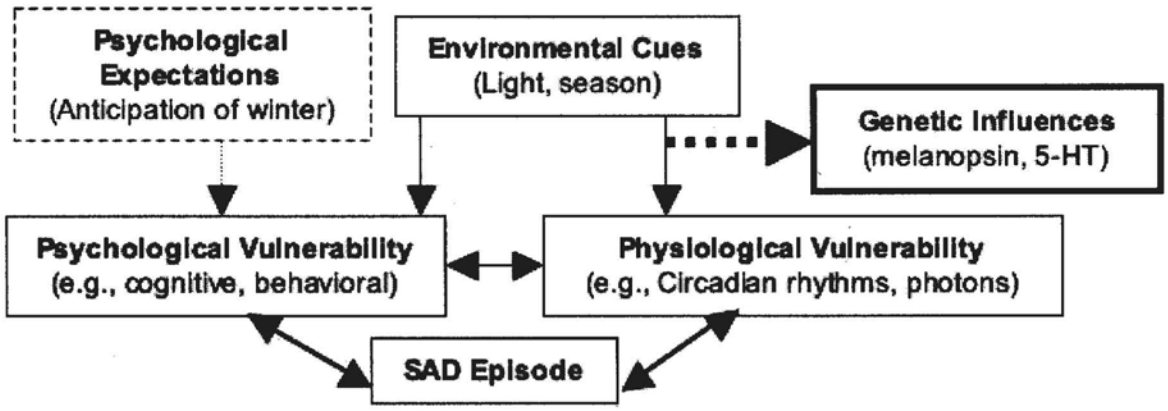
*The Integrative, Cognitive-Behavioral Model*

Rohan (2002) has created a conceptual model combining physiological vulnerabilities with specified psychological vulnerabilities to SAD (Figure 1). The integrative model is an expansion of Young's dual vulnerability model, which was the first to propose both

psychological and physiological vulnerabilities in SAD (Young, 1999). According to this expanded model, genes, including melanopsin, may mediate the physiological response to environmental cues such as light in SAD. The psychological vulnerability consists of cognitive (Beck, 1967; 1976) and behavioral factors such as maladaptive schemas, negative automatic thoughts, rumination, and reduced response-contingent positive reinforcement. In addition, the model proposes that light- and season-specific cognitions play a role in the psychological vulnerability to SAD (Rohan, Sigmon, & Dorhofer, 2003).

A learned association between winter season and low light cues and depression influences a conditioned anticipation of winter that is proposed to activate the psychological vulnerability. Alternatively, environmental changes such as declining light levels may precipitate an episode of SAD by activating a reciprocal loop between psychological and physiological factors. The specific proposed psychological factors include behavioral inhibition that leads to reduced interaction with the environment and fewer opportunities for positive reinforcement through physical and social activities, according to Lewinsohn's theory of behavioral disengagement (Lewinsohn, 1974, 1985). Individuals with SAD may hold dichotomous core beliefs that are negative about low light and winter season and overly positive about bright light and summer season in addition to maladaptive core beliefs, attitudes, and automatic thoughts seen in nonseasonal depression (Beck, 1967, 1976). Rumination is an additional cognitive vulnerability and involves focusing on the causes and consequences of depressed mood, a cognitive response style that predicts future depression (Nolen-Hoeksema, 1987). A critical component of the integrative model is that purely biological explanations for SAD are insufficient to describe the onset, maintenance, and etiology of SAD.

*Figure 1.* Integrative model for SAD.




Note. The Genetic Influences component was added to Rohan's (2002) model to provide a conceptual framework for the present study.

*Genetic factors as part of the overall model.* For the purposes of the present study, we propose to test for the involvement of one genetic factor in the biological vulnerability to SAD. Moreover, it is proposed that genetic factors are just one component of a multi-factorial etiological model for SAD. The psychological and environmental factors described above in the integrative hypotheses of SAD are the other factors, beyond biological factors, that are hypothesized to contribute to variance in SAD onset, maintenance, exacerbation, and recurrence. Below, previously proposed biological hypotheses for SAD are reviewed. Most of these biological hypotheses for SAD are consistent with the present study hypothesis that melanopsin may be one gene that contributes to the risk of developing SAD.

*How physiological dysregulation may lead to SAD.* With all conceptual models, it is important to specify the mechanism by which a physiological risk factor can lead to depression. All three models discussed suggest a role for physiological dysregulation in contributing to depression in SAD. Sequence variation in melanopsin may affect the phototransduction efficiency of the photopigment, thereby raising the threshold of light input

such that low winter light levels fall below this threshold in SAD. This may lead to a decrease in already low winter levels of serotonin in the brain, affecting the circadian clock and stress response system leading to symptoms of SAD. Specifically, circadian dysregulation is expected to lead to fatigue and changes in sleep and eating patterns. Alterations in the stress response system due to regulation by 5-HT could lead to difficulties with concentration and/or changes in psychomotor activity. Sequence variations in genes for serotonin transporters or receptors may interact to exacerbate dysregulation of the circadian and stress response system as both systems are innervated by serotonergic pathways. Finally, symptoms including sleep and appetite changes, fatigue, difficulty concentrating, and psychomotor changes may comprise a vulnerability that interacts with psychological factors leading to low mood and/or a loss of interest in daily activities, hallmark symptoms of depression, and possibly suicidal ideation.

Also analogous to non-seasonal depression, different hypotheses for SAD are not mutually exclusive, and an integrative approach including monoamines, genes, and psychological and environmental factors is likely to best explain SAD. In light of these multiple factors, the integrative, cognitive-behavioral model for SAD (Rohan, 2002) is further developed by proposing that genetic variations mediate the environmental cues of light and season in a physiological vulnerability to SAD (See Figure 1, above). Other potential molecular mechanisms (i.e., genes) for a biological vulnerability to SAD may mediate the environmental precipitants of SAD, comprising the link between physiological dysregulation and depression in SAD.

*The Photoperiod Hypothesis*

The photoperiod hypothesis is one way of understanding how light input, circadian rhythms, and SAD may be related. Photoperiod is the number of hours from sunrise to sunset (Dunlap, Loros, & DeCoursey, 2004). Organisms use environmental light information (i.e., timing of light onset and offset and light intensity) for non-visual functions such as determining time of day. Photoperiod is determined exclusively by two factors: day of year and latitude. Early SAD researchers postulated that SAD would be more common at extreme latitudes because of the progressively shorter winter photoperiods as distance from the equator increases. The photoperiod hypothesis was proposed initially by Rosenthal et al. (1984). Indeed, initial epidemiological surveys at varying latitudes in the U.S. found evidence for increasing rates of SAD with more Northern latitudes, ranging from 1.4% in Florida to 9.7% in New Hampshire (Mersch, Middendorp, Bouhuys, Beersma, & van den Hoofdakker, 1999), supporting the theory that decreasing photoperiod leads to increased risk of SAD. Subsequent studies found that latitude accounts for a smaller percentage of variance in SAD prevalence rates outside of North America (Molin, Mellerup, Bolwig, Scheike, & Dam, 1996). Individuals with a deficiency in responding to non-visual light input, differences in the experience of natural or artificial light, or differences in the function of the circadian clock could lead individuals with SAD to be more vulnerable to SAD as photoperiod decreases with increasing latitude (Sohn & Lam, 2005). With regard to the present study hypothesis, polymorphisms in the gene for melanopsin could lead to an abnormally low sensitivity to non-visual light input that may render individuals more vulnerable to SAD in low light environments like extreme latitudes or winter season, both of which are characterized by shortened photoperiods.

*Environmental Correlates of SAD*

Genetic factors often interact with environmental factors in contributing risk of a disorder. Therefore, data on the environmental factors thought to be operating in SAD are reviewed here. Studies have found a correlation between some climatic variables and SAD, suggesting an environmental factor in SAD etiology. Environmental light-related parameters that vary with season include photoperiod and the intensity of natural light, which can be measured using daily minutes of sunshine that produce a detectable shadow, cloud cover, and global radiation. Depression severity in individuals with SAD, as measured by the Beck Depression Inventory (BDI; Beck, Rush, Shaw, & Emery, 1979), correlates negatively with photoperiod, overall intensity of available light (global radiation and total minutes of sunshine), and temperature (Molin et al., 1996). One study correlated photoperiod and intensity of light (as measured by global radiation) with SAD episode onset (onset of first SAD symptom in a given year). Only photoperiod emerged as a significant predictor of SAD episode onset (Young, Meaden, Fogg, Cherin, & Eastman, 1997). These data suggest that individuals with SAD have an abnormal response to shortening photoperiod in fall and winter, a response that could be the result of having too few functional non-visual photoreceptors. With an insufficient number of non-visual photoreceptors, low light levels in winter could fall below the threshold required for proper neural signaling in the non-visual light input pathway.

*Circannual Rhythms and Photoperiod*

Both circadian and circannual rhythms are entrained through non-visual light input to the brain, and are therefore important issues to review with respect to the present study of melanopsin in SAD. In addition to the hypothesized role of photoperiod in SAD, some

propose that SAD is due to circannual or yearly rhythms that are controlled by the circadian clock. SAD is characterized by both circannual (yearly) rhythms and abnormalities in circadian (daily) rhythms for sleep, appetite, mood, energy or alertness, melatonin release, and core body temperature (APA, 2000; Teicher et al., 1997), as reviewed below. Photoperiod is a cue used by animals with seasonal changes in behavior to entrain circannual rhythms (Dunlap et al., 2004). Unlike individuals without SAD, the seasonal presentation of depression symptoms in SAD suggests that individuals with SAD may present with disturbances in circannual rhythms related to mood and behavior or may experience biological changes in response to changing photoperiod (Wehr, 2001). Circadian rhythm disregulation in SAD is cited as support for a circadian explanatory theory of SAD (Sohn & Lam, 2005). The two circadian hypotheses for SAD are the photoperiod hypothesis described above and the phase-shift hypothesis described below. Photoperiod is used by animals to determine season in order to initiate adaptive responses to the seasons (Dunlap et al., 2004). Some SAD researchers suggest that humans with SAD retain seasonal rhythms in mood and behavior while individuals without SAD do not have seasonal rhythms. This suggests that individuals with SAD may have differences in the processing of non-visual light information when compared to individuals without SAD, and provides further support for a role for melanopsin in SAD.

*SAD resembles hibernation seen in other mammals.* The timing of hibernation is controlled in mammals by the circadian clock, and due to the similarities between SAD symptoms and hibernation in mammals, may help us understand the role of circannual rhythms in humans. Some individuals with SAD experience symptoms similar to the behaviors observed in hibernating animals. Major depression can include the symptoms of

depressed mood; loss of interest in activities; changes in appetite, weight, and sleep; psychomotor retardation or agitation; fatigue or loss of energy; feelings of guilt or worthlessness; difficulty concentrating; and recurrent suicidal ideation (APA, 2000). The atypical depression symptoms common in SAD are similar to seasonal changes in hibernating animals, including decreased activity level and social interaction, hypersomnia, increased appetite, weight gain, reduced metabolism, and decreased reproductive behavior (Wehr, 1999). The similarity between seasonal presentation of behavioral changes in SAD and seasonal animal behavior suggests a common biological mechanism (Wehr, 2001).

*Photoperiod and Melatonin*

The hormone that conveys information about photoperiod to the body, and may be involved in depression symptoms is melatonin. Melatonin is a neuroendocrine signal of photoperiod released from the pineal gland that is regulated by the circadian clock (Dunlap et al., 2004). The most predictable environmental parameter associated with the change of season is photoperiod (Provencio, 2004), the length of time from dawn to dusk. In animals, changes in photoperiod signal the pineal gland to lengthen or shorten the duration of melatonin release at night which, in turn, produces changes in the reproductive drive, activity level, sleep, feeding, weight, and metabolism (Wehr et al., 1995). Mice require melanopsin and functional rods and cones to acutely suppress melatonin synthesis in response to light (Panda et al., 2003). It is possible that humans with a genetic variation in melanopsin also may fail to fully respond to light with acute melatonin suppression and experience a lengthened duration of melatonin synthesis when light levels are lower in the winter. A lengthened duration of nocturnal melatonin release in winter could be one mechanism underlying the seasonal presentation of SAD symptoms, analogous to the mechanism for

seasonal behaviors in rodents. This involvement of melatonin in signaling season may be the link between non-visual light input and resultant depression in SAD.

*A circadian signal of change of season.* Wehr et al. (2001) conducted a study providing some support for the photoperiod hypothesis of SAD. In individuals with SAD, the duration of active nocturnal melatonin release was longer in winter than in summer; however, healthy controls did not show winter-summer differences in nocturnal melatonin release (Wehr et al., 2001). Using detectable melatonin in plasma, only men with SAD evidenced a longer duration of nocturnal melatonin release in winter to summer, whereas women with SAD and healthy controls did not differ from winter compared to summer (Wehr et al., 2001). A lengthened melatonin release duration in some individuals with SAD suggests that they track seasonal change physiologically through a "circadian signal of change of season" (p. 1108, Wehr et al., 2001). Individuals with SAD who experience this seasonal change in melatonin profile may respond differently to environmental light cues in the naturalistic environment compared to individuals without SAD (Wehr et al., 2001). It is possible that artificial light levels (e.g., household lights during dark hours) are of insufficient intensity or duration to normalize the duration of nocturnal melatonin release in certain individuals with SAD. As one part of the environmental light input pathway, melanopsin may underlie this type of seasonal change in SAD.

*The Non-Visual Light Input Pathway*

Defects in the non-visual light input pathway may be the mechanism by which melanopsin is involved in SAD, so the pathway is described here. It is important to the present study to understand that melanopsin is only one of a handful of molecules through which non-visual light information is conveyed. We propose that SAD is due to multiple risk

factors, potentially caused by mutations in multiple genes. However, the present study tests only one candidate gene for SAD. Other molecular components of the non-visual light input pathway, other than melanopsin, could hypothetically be involved in SAD and result in a phenocopy. A phenocopy is a situation in which two people have the same phenotype or trait (i.e., SAD) but have differing underlying genetic causes. Genes for molecular components of the melanopsin signaling pathway could lead to SAD by increasing the threshold of light input necessary for euthymic functioning. Therefore, a brief description of the role of potential candidate genes for SAD involved in the melanopsin-based signaling pathway follows. Molecular components of the non-visual light input pathway may be involved in SAD for the same proposed reasons that melanopsin is involved in SAD.

*Signal transduction conveys non-visual light information to the brain.* Non-visual light information is conveyed to the brain through a signaling pathway. Signal transduction is a process by which the sensory cells convert one kind of signal, such as light, to a biologically relevant signal, such as neuronal action potentials, using ordered sequences of biochemical reactions inside the cell. Signaling pathways comprise this ordered sequence of biochemical reactions, and involve the following components: receptors, second messengers, and effectors. Opsin-based photopigments, such as the melanopsin molecule, react to light by changing their structural conformation. This change in shape initiates a sequence of biochemical reactions. Upon photoactivation, photopigments associate with and subsequently activate heterotrimeric G-proteins, which are a type of second messenger. Upon activation, G proteins experience a conformational change that triggers the dissociation of one of the G protein subunits. These dissociated subunits can then interact with effector proteins such as enzymes. Enzymes are proteins, such as phospholipase C (PLC) or protein

kinase C (PKC), that catalyze or accelerate reactions between selective substrates. The products of these enzymatic reactions, in turn, can directly or indirectly gate membrane localized ion channels, resulting in changes in membrane potential. These changes in membrane potential are the informational currency of the nervous system and the ultimate endpoint of the signal transduction process.

*Molecules involved in the melanopsin signal transduction cascade.* Melanopsin triggers a $G_q$ protein-dependent signaling cascade that activates PLC, resulting in the hydrolysis of membrane phosphoinositides. This is the same signaling pathway initiated by the opsin-based photopigments of invertebrates (Kumbalasiri, Rollag, Isoldi, de Lauro Castrucci, & Provencio, 2006). Activation of the phosphoinositide pathway by melanopsin ultimately leads to increase in calcium levels within the cell (Isoldi, Rollag, Castrucci, & Provencio, 2005; Kumbalasiri et al., 2006; Sekaran, Foster, Lucas, & Hankins, 2003), which changes the electrical potential and allows ipRGCs to communicate with cells in the brain.

*Specific molecular components of the signaling cascade.* Molecular components of the melanopsin-based signaling pathway that have been identified or implicated at this time include: the $G_q$ family of G-proteins; enzymes PLC and PKC; the canonical *transient receptor potential* subfamily (TRPC) of cation channels, which are involved in electrical currents in the cell; arrestin (Isoldi et al., 2005; Melyan et al., 2005; Panda et al., 2005; Qiu et al., 2005); and inositol 1,4,5-triphosphate ($IP_3$) receptors. Arrestin is a type of protein that can inhibit signaling when bound to a G protein-coupled receptor (GPCRs). Based on research in mammalian cell lines artificially expressing melanopsin, melanopsin initiates a G-protein signaling cascade when exposed to light (Melyan et al., 2005; Qiu et al., 2005), that subsequently activates the phosphoinsositide pathway (Kumbalasiri et al., 2006; Qiu et al.,

2005). PLC-β is a member of the PLC isozyme family that is preferentially activated by the α subunit of $G_q$ (Panda et al., 2005; Rhee, 2001). PLC catalyzes a reaction intracellularly that is thought to lead to an increase in calcium in the cell (Panda et al., 2005; Rhee, 2001) through TRPC channels. These molecules are also candidate genes for SAD.

*Data from animal models suggests specific molecules are involved.* In *Xenopus* cells, light activates melanopsin, leading to an increase in calcium intracellularly (Isoldi et al., 2005; Panda et al., 2005). In mammals, PLC-β4 is found in the retina (Rhee, 2001), and data strongly suggests that melanopsin, when activated by light, specifically activates a $G_q/G_{11}$ subtype of G protein, which in turn activates an isoform of PLC-β (Panda et al., 2005; Qiu et al., 2005). It is clear that the stimulation of ipRGCs by light leads to an increase of intracellular calcium and depolarization of the cell (Sekaran et al., 2003). In other systems, TRP channels mediate light-induced influx of $Ca^{2+}$ and Na+ into the cells, underlying the observed depolarization of photoreceptors in response to light (Montell, 2005). TRPC3 channels are preferentially activated by $G_q/G_{11}$ proteins (Qiu et al., 2005), and coexpressing TRPC3 channels and melanopsin in a human embryonic kidney cell line (HEK293) led to an intracellular $Ca^{2+}$ increase similar to that seen in ipRGCs. Therefore, TRPC3 channels are believed to potentially carry the light-activated depolarizing current in the melanopsin signaling pathway (Qiu et al., 2005). However, light-activation of melanopsin also leads to increases in intracellular inositol-triphosphate ($IP_3$), which liberates sequestered intracellular $Ca^{2+}$ through $IP_3$-gated $Ca^{2+}$ channels localiized to the membrane of the endoplasmic reticulum, an intracellular $Ca^{2+}$ store. This increase in intracellular $Ca^{2+}$ initiates other signaling pathways, and activation of PKC, which is likely to phosphorylate other components of the melanopsin signaling pathway (Isoldi et al., 2005). Phosphorylation can

switch on or off components of a signaling cascade. Therefore, the molecules PKC, $IP_3$, TRPC3, $G_q/G_{11}$ proteins, and PLC-β4 are parts of the melanopsin signaling pathway that could play a role in SAD, similar to that which we propose for melanopsin itself.

*The potential role of arrestin in signal transduction.* Other opsin-based photoreceptors such as rhodopsin have components including G protein receptor kinases, arrestin, and other molecules that serve to regulate the signaling cascade. Arrestins bind specifically to active (phosphorylated) GRCRs, to arrest or reduce signaling by these receptors. If homologues of these regulatory molecules are present in ipRGCs, they too would be candidate genes for SAD.

*PACAP is involved in non-visual responses to light.* The neurotransmitter pituitary adenylate cyclase-activating polypeptide (PACAP), and glutamate are known to be neurotransmitters in the retino-hypothalamic track conveying non-visual light information from ipRGCs to the circadian clock (Hannibal, 2006). PACAP is found exclusively in the neural pathway from the retina to the SCN in the hypothalamus (Hannibal, 2006), however, glutamate is found in many pathways.

This description of the molecules involved in the melanopsin-based signaling pathway outlines the genes other than melanopsin that may be involved in SAD. If melanopsin itself does not appear to be a risk factor for SAD, the hypothesis supporting melanopsin's role may be applicable to the above-mentioned molecular components of the non-visual light input pathway.

*Retinal Subsensitivity in SAD*

Another hypothesis to explain SAD is that the retina of individuals with SAD may be less sensitive to light such that low winter light levels lead to sub-threshold levels of light

input to the brain. Evidence suggests that individuals with SAD may seek increased exposure to available outdoor light (Graw, Recker, Sand, Krauchi, & Wirz-Justice, 1999), possibly due to diminished sensitivity of the retina. The retinal sensitivity hypothesis proposes that the retina, as a whole, is less sensitive to environmental light cues in SAD, and that this insensitivity may result from abnormalities in rods, cones, or other retinal photoreceptors (Hebert, Dumont, & Lachapelle, 2002) such as melanopsin-containing retinal ganglion cells. Normally, under low light conditions, the retina increases its sensitivity to light in order to maintain proper functioning. This up-regulation of sensitivity may not occur in individuals with SAD (Hebert et al., 2002). Specifically, SAD patients may have a decreased sensitivity to the amount of light they receive year-round. If so, then available photons for retinal absorption in winter may fall below the threshold for normal function of the non-visual light input pathway.

*Clinical findings supporting retinal subsensitivity in SAD.* Some clinical research supports diminished retinal sensitivity in SAD. Electrooculographic (EOG) ratios, an objective measure of the electrical response of the entire retina to light, appear to be lower in SAD cases than in controls, although there is significant overlap between the groups in EOG ratios (Lam, Beattie, Buchanan, Remick, & Zis, 1991). Healthy individuals displayed higher EOG ratios in winter than in summer, whereas individuals with SAD did not demonstrate this seasonal variation in retinal response (Ozaki, Rosenthal, Myers, Schwartz, & Oren, 1995). Another study found no difference between individuals with SAD and controls in retinal contrast sensitivity (sensitivity to contrasting light and dark stimuli) or in visual evoked electroencephalograph (EEG) responses (Murphy et al., 1993). In contrast, other researchers have found that individuals with SAD, compared to healthy controls, exhibit supersensitivity

of the retina in the winter (Terman & Terman, 1999). In a study of individuals with subsyndromal SAD (S-SAD), the S-SAD group, but not healthy controls, evidenced a decrease in winter retinal sensitivity (Hebert et al., 2002). Inconsistent results in research on retinal sensitivity may reflect different types of retinal responses measured (e.g., EOG vs. visual evoked EEG vs. self-report of detection of visual stimuli by participants). As melanopsin containing cells are only a small percentage of one type of cell in the retina, ganglion cells (Provencio et al., 2002), deficits in the sensitivity of this one cell would not be observable in whole-retina measurements of sensitivity.

*Naturalistic studies of retinal subsensitivity.* Further evidence for retinal subsensitivity in SAD comes from naturalistic studies comparing light exposure profiles in SAD vs. non-SAD individuals across the seasons. Women with SAD spend more time outdoors in the summer, but not in the winter, than non-SAD controls (Graw et al., 1999). This finding implies that individuals with SAD have an increased need for light and increase their light input in the summer by going outside, but may be unable to do this in the winter because of winter weather, low temperatures, later dawn, and earlier dusk, leading to SAD. A greater need for light, possibly resulting from a decreased retinal sensitivity, would support a role for melanopsin in SAD because individuals with fewer functional photoreceptors may experience reduced biologic sensitivity to light. Individuals with fewer functional melanopsin-containing cells may also show decrements in retinal sensitivity similar to those identified in SAD.

*Seasonality and Depression*

Because SAD is characterized by a seasonal pattern of depression, and any associated genetic factors may be involved in either seasonality, depression, or both factors, the

distinction between the constructs of seasonality and depression are discussed here. Behaviors and disorders in addition to depression demonstrate a seasonal variation in severity or prevalence, including drinking behavior and alcoholism (McGrath & Yahia, 1993), bulimia nervosa (BN; Blouin et al., 1992), anorexia nervosa (AN; Fornari et al., 1994), panic disorder (Marriott, Greenwood, & Armstrong, 1994b), schizophrenia and schizoaffective disorder (Sperling, Barocka, Kalb, Suss, & Katalinic, 1997). A number of disorders are highly co-morbid with SAD diagnosis including bulimia nervosa (Lam, Solyom, & Tompkins, 1991), binge eating disorder (Rohan, Roecklein, Nguyen, Johnson, & Handal, 2004), generalized anxiety disorder, simple phobia, and social phobia (Levitt, Joffe, Brecher, & MacDonald, 1993). On the other hand, some disorders do not appear to be associated with seasonal variation such as obsessive-compulsive disorder (Yoney, Pigott, L'Heureux, & Rosenthal, 1991). Certain psychological disorders do show seasonal patterns, and some do not, suggesting that seasonality and depression may be different constructs, and may have different underlying pathophysiologies.

*Depression and seasonality may be separate constructs.* It is possible that depression and seasonality are separate constructs that overlap in SAD alone (Lam et al., 2001). If that is the case, many individuals with psychological disorders can be expected to have a seasonal variation, if those individuals carry the seasonality vulnerability and a vulnerability for another psychological disorder. However, it is also possible that there is a specific reason why individuals who have the tendency to vary in mood and behavior across the seasons preferentially display behaviors of one type (i.e., low libido, depressed mood, hyperphagia, hypersomnia, psychomotor retardation, behavioral inhibition) as opposed to alternate behavioral profiles (i.e., psychotic behavior). The seasonal exacerbation and co-morbidity

data above suggest that the tendency to vary in mood and behavior across seasons is not specifically associated with depressive behaviors, but may be a general phenomenon in psychopathology (Marriott et al., 1994b).

However, some data suggest that disorders with symptoms that overlap with SAD symptoms, or behaviors that are common to SAD and another disorder are the most likely to vary across seasons. For example, individuals with AN are less likely than individuals with BN to have seasonal changes in mood and behavior (Fornari et al., 1994), individuals with BN are likely to display both binge and restraint eating behaviors (Berman, Lam, & Goldner, 1993), and individuals with SAD are likely to binge but unlikely to display restraint eating behaviors (Krauchi, Reich, & Wirz-Justice, 1997). It is possible that the binge eating behavior in BN and binge eating disorder shares a common etiology with the hyperphagia and carbohydrate craving reported in SAD, namely that these behaviors are more pronounced in winter due to changes in photoperiod (Wehr, 2001). Seasonal changes that are similar in some humans and some other mammals include loss of libido, behavioral inhibition, social withdrawal, hypersomnia, hyperphagia, and weight gain (Wehr, 2001). It is possible that the co-morbidity of social phobia and SAD is due to a common etiological factor behind social withdrawal behaviors seen in both disorders, however, it is unclear why generalized anxiety disorder and specific phobias might be co-morbid with SAD. Similarly, it is unclear why schizophrenia may have a seasonal exacerbation. It is currently unknown if sexual disorders have a seasonal variation, although some evidence suggests that deviant sexual behavior is more common in summer (Bicakova-Rocher, Smolensky, Reinberg, & De Prins, 1985), and this is consistent with the decrease in libido seen in winter in SAD. A common etiology for SAD and panic disorder has been proposed, specifically that the avoidance of public places

in individuals with agoraphobia may lead to increased home confinement and an even greater decrease in both environmental light input and a reduced rate of positive reinforcement for behavioral activation in winter (Marriott et al., 1994b). A common genetic etiological factor for SAD and alcoholism has been proposed, specifically that a polymorphism in a gene related to serotonin (5-HTTLPR) is associated with both SAD and alcohol abuse and dependence (Sher, 2002). In each case mentioned above, it is not clear if one disorder leads to SAD, SAD leads to other disorders, or disorders share a common etiology. One method for resolving this dilemma would be to identify genetic risk factors that segregate with specific symptoms or symptom clusters across disorders. Therefore, the present study will test associations between the melanopsin gene and both seasonality and SAD diagnosis, as well as preliminarily investigate associations with depressive symptom severity.

*Circadian Abnormalities in SAD*

Further evidence linking melanopsin and SAD comes from observed circadian rhythm abnormalities in SAD. Circadian abnormalities in mood, sleep, core body temperature, and neuroendocrine secretion have been reported in SAD (Schwartz et al., 2000). Circadian sleep disturbances include hypersomnia, increased sleep latency, and an increase in slow-wave sleep (Avery et al., 1997; Dahl et al., 1993; Khalsa, Jewett, Duffy, & Czeisler, 2000; Shanahan & Czeisler, 1991). Melanopsin-containing cells send projections to the central circadian clock in the hypothalamus, the suprachiasmatic nuclei (SCN; Hattar et al., 2002), suggesting that variations in melanopsin function could plausibly underlie circadian abnormalities observed in SAD.

*Methodological difficulties in identifying circadian rhythm abnormalities.* A confound in interpreting circadian rhythm aberrations is that these rhythms are vulnerable to

perturbation by activities such as sleep and waking behavior in addition to differences in environmental light information. For example, a delay in sleep onset may be due to either a delay in the central clock, a delay in the sleep homeostat, delayed sensitivity to the sleep homeostat, or masking of sleep onset by late night activity. Therefore, the timing of sleep may not directly reflect the phase of the circadian clock and can be influenced by recent sleep history and activity levels. Some rhythms, including core body temperature and melatonin release, are more closely linked to the circadian clock and are relatively less affected by activity rhythms, although the effects of activity, posture, caloric intake, and sleep must be carefully controlled when measuring these rhythms (Dahl et al., 1993). Therefore, data on rhythms of core body temperature and melatonin release are the most reliable for detecting circadian rhythm abnormalities in SAD, and form the basis for the investigation into non-visual light input in SAD.

*Phase-shift Hypothesis*

Hypotheses such as the circadian rhythm hypothesis and the phase-shift hypothesis are used to provide plausible roles for melanopsin and non-visual light input in SAD. The second circadian-related hypothesis to explain SAD is the phase-shift hypothesis (Sohn & Lam, 2005). The phase-shift hypothesis proposed by Lewy et al. (1987), suggests that SAD results from internal circadian rhythms being delayed relative to environmental time or relative to other rhythms like the sleep-wake rhythm. Some studies have found that rhythms in core body temperature, cortisol profile, and dim light melatonin onset (or DLMO) are phase-delayed (i.e., the timing of a physiological function occurs later than expected) in some individuals with SAD and can be advanced with morning light therapy (Avery et al., 1997; Czeisler et al., 1989). However, one study found no difference between SAD patients and

controls in nocturnal melatonin release onset time (Wehr et al., 2001). Instead, Wehr et al. (2001) found that melatonin release offset time was delayed in patients with SAD in winter compared to summer, but that this phase delay was not found in healthy volunteers. The phase-shift hypothesis of SAD is controversial because many studies have not found phase-shifts in individuals with SAD (Sohn & Lam, 2005). One explanation for this may be that there are subsets of individuals with SAD, some of whom have phase-delays, and some of whom have different biological mechanisims in their SAD vulnerability (Sohn & Lam, 2005). One mechanism by which melanopsin could be linked to the etiology of SAD is that certain variants of the melanopsin protein could be less efficient and lead to a delay in circadian rhythms.

*Antidepressant Effects of Light on SAD*

The demonstrated efficacy of bright light therapy in treating SAD provides further support for a role for non-visual photoreception in SAD. Bright light therapy is the established and best available treatment for SAD (Terman et al., 1989). A recent meta-analysis of light therapy trials for SAD found an average effect size of 0.84 (95% CI = 0.6-1.08), which is equivalent to effect sizes found in antidepressant medication trials for depression, and an average odds ratio for remission of 2.9 (95% CI = 1.6-5.4), indicating that light therapy is about three times more likely than credible placebos to produce full remission (Golden et al., 2005). Across studies, 53% of individuals with SAD overall and 43% of moderate to severe cases remitted with a supervised trial of light therapy (Terman et al., 1989). Therefore, light therapy is a good, but not completely effective treatment for SAD.

*Light is the most important time cue in humans.* Light resets the internal clock even when individuals are isolated from other time cues, demonstrating that light is the primary

environmental cue used by the circadian clock to determine time of day (Magnusson & Boivin, 2003). Available light levels in the winter may be too low for euthymic functioning in individuals at risk for SAD, a tenet of the photoperiod and phase-shift hypotheses of SAD. However, the potential mechanisms underlying light therapy's effects on SAD may include restoring proper functioning of the circadian clock or other non-circadian, non-visual effects that light may have. Given melanopsin's involvement in circadian photoentrainment and other non-visual responses to light, melanopsin may play a role in light therapy's antidepressant effects on SAD.

*Parameters of non-visual light information.* The optimal parameters for light therapy might indicate what type of non-visual light information is most important in SAD. Because light has a variety of effects on the body, researchers have attempted to manipulate several light-related parameters to determine the optimal prescription for SAD, such as timing, intensity, duration, and wavelength (Lee, Chan, Paterson, Janzen, & Blashko, 1997; Oren et al., 1991). Research using light therapy regimens designed to shorten the nocturnal duration of melatonin release, to simulate a summer-like photoperiod, to phase-advance circadian rhythms, and to increase the number of photons delivered to the retina is described below. As described below, the details of how light therapy has been used in treating SAD and the parameters that have been most effective suggest a role for melanopsin in mediating the antidepressant effects of light on SAD.

*Morning light to phase-advance circadian rhythms.* The antidepressant effects of light on SAD may be related to light's ability to advance abnormally delayed circadian rhythms in SAD as proposed by the phase-shift hypothesis (Lewy, Sack, Singer, & White, 1987). Terman et al.'s (1989) quantitative review of light therapy's efficacy concluded that

light therapy administered in the early morning had a higher remission rate (53%) relative to light therapy in the evening (43% remission rate) or midday (32% remission rate). Most head-to-head comparisons have found that morning administration of light is more effective than evening light for SAD, leading researchers to recommend that light therapy be scheduled immediately upon awakening (Meesters, Jansen, Beersma, Bouhuys, & van den Hoofdakker, 1995; Wehr et al., 1986; Wirz-Justice et al., 1993). However, some trials have shown that evening light is efficacious for SAD, and there is no evidence that light therapy in the evening makes SAD symptoms worse.

Whether morning light therapy or dual morning-evening administration is superior remains open to debate. Morning light therapy did not differ from morning-plus-evening (52% remission rate) in Terman et al.'s analysis. Lee et al.'s (1997) meta-analysis found that the morning-plus-evening combination demonstrated greater effect sizes ($d = 2.09$) than morning alone ($d = 1.74$), evening alone ($d = 1.35$), or mid-day ($d = 1.27$) light administration. Given that it is a more recent meta-analysis and included 40 light therapy trials, the Lee et al. (1997) study may have conducted the most statistically powerful test comparing light therapy regimens. Because combined morning and evening administration of light are most important for photoentrainment in animals, superior efficacy of morning and evening light combined for SAD may indicate a similar pathway to photoentrainment between humans with SAD and animals via melanopsin containing circadian photoreceptors.

*Phase-shifting effects of light therapy.* Terman et al. (2001) investigated the antidepressant effects and phase-shift responses to morning and evening light in individuals with SAD. Results suggest that there may be a relationship between the magnitude of phase-advance caused by morning light and the degree of antidepressant response to light therapy

(Terman, Terman, Lo, & Cooper, 2001). However, total improvement in depression scores did not differ between groups receiving morning versus evening light.

Heterogeneity in the findings of light therapy studies suggests heterogeneity in the pathogenetic role of light among individuals with SAD. Melanopsin may be one of a number of molecules involved in the non-visual light input system that could underlie differing vulnerabilities for sub-groups of individuals with SAD. Alternatively, different variations within the melanopsin gene may confer different functional consequences that may explain the heterogeneity of findings across light therapy trials. In addition, identifying a mechanism for a biological vulnerability to SAD may explain why a signficant minority (47%) of individuals with SAD does not remit with light therapy (Terman et al., 1989).

*Potential Mechanisms for a Biological Vulnerability to Depression*

A review of biological models for depression and recent developments is presented below, and is integrated into the hypotheses of SAD etiology, and is important when evaluating the consistency between established hypotheses of SAD etiology and the hypothesized role for melanopsin in SAD in the present study. Historically, biological explanations for depression have focused on the monoamine signaling pathways of serotonin (5-HT), norepinephrine (NE), and dopamine (DA; Thase, Jindal, & Howland, 2002). Current models integrate the stress response system of the hypothalamic-pituitary-adrenocortical (HPA) axis, the effects of monoamines on gene activity in neurons and neurogenesis (the creation of new neurons), neuronal plasticity and survival, and the role of sleep with the role of monoamines in depression (Hayley, Poulter, Merali, & Anisman, 2005; Thase et al., 2002). More broadly, psychosocial factors are thought to interact with chronic stress and genetic vulnerability factors to affect neural function and survival, leading to depression

(Hayley et al., 2005). Abnormalities in these central nervous system (CNS) processes tend to co-aggregate in severe or chronic depression, although they are often not specific to depression, and there is heterogeneity in the presentation of biological markers of dysregulation (Thase et al., 2002). CNS dysregulation may constitute a vulnerability to depression or illness in general, that, when paired with severe or chronic stress during development, leads to depression (Thase et al., 2002).

*Dysregulation of monoamine signaling pathways.* Monoamines are hypothesized to be involved in depression and SAD, and also vary across the seasons, indicating they may be light or photoperiod dependent. Chronic stress has been shown to reduce levels of DA (Willner, 1995), 5-HT (Lopez, Liberzon, Vazquez, Young, & Watson, 1999; Weiss & Kilts, 1998), and NE (Thase et al., 2002) in the brain, and these decreases have been correlated with rodent behavior suggestive of depression such as behavioral inhibition, weight loss, and decreased sleep (Weiss & Kilts, 1998; Willner, 1995). Findings of specific types of dysregulation of the interactive monoamine pathways are described below as they relate to depression.

*Serotonin in depression.* 5-HT pathways are involved in normal behaviors that are disrupted in depression including appetite regulation (Thase & Howland, 1995), circadian regulation of sleep, body temperature, and HPA axis function (Bunney & Bunney, 2000; Duncan, 1996), affiliative behavior (Insel & Winslow, 1998), and goal directed and appetitive behavior (Thase et al., 2002). Research on 5-HT levels and 5-HT-related gene variants has linked neurotransmission of this monoamine to suicide (Maes & Meltzer, 1995), bipolar disorder, and depression-related personality traits such as neuroticism as measured by the NEO Personality Inventory, but only weakly with Major Depressive Disorder (MDD)

diagnosis per se (Kalia, 2005; Levinson, 2005). The lack of findings associating 5-HT with depression diagnosis and additional research suggest that 5-HT may be involved in stress reactivity as opposed to depression per se (Caspi et al., 2003) Neuroticism may mediate stress sensitivity and genetic risk through either the generation of interpersonal stressors as well as reactions to stressors in depression (Kendler, Gardner, & Prescott, 2003). Importantly, 5-HT and NE interact in a counterbalancing way to modulate the HPA axis in response to stress, as further described below.

*Neurobiological response to stress.* Monoamines and stress may interact in leading to depression, and are therefore important issues with respect to the present study. Responding to stress is important in depression because it is well established that stressful life events and vulnerability to stress are risk factors for depression (Akiskal, 1985; Paykel, 1979). 20-40% of depressed outpatients and 60-80% of depressed inpatients have hypercortisolism or increased levels of cortisol (Thase et al., 2002), the principal hormone released from the adrenal gland in response to stress (Kandel, Schwartz, & Jessell, 2000). The HPA axis is stimulated by NE and inhibited by 5-HT, modulating stress-elicited output of neuropeptides from the hypothalamus, pituitary, and adrenal gland, with cortisol levels inhibiting upstream steps in the HPA axis in an autoregulatory negative feed-back loop (Thase et al., 2002). One neuropeptide, CRH, stimulates the locus ceruleus (LC), which stimulates the hypothalamus to release more CRH, establishing a positive feedback loop under conditions of sustained stress, and this may be the mechanism behind hypercortisolism in a sub-set of individuals with depression. Hypercortisolism may be causative in depression because there is evidence that chronically high cortisol levels lead to cell death in the

hippocampus (Hayley et al., 2005). Hippocampal atrophy is thought to cause the cognitive deficits and negative mood in depression.

*Neurogenesis, neuronal plasticity and survival.* Neuronal plasticity, or the ability of neurons to change synaptic connections or establish new dendritic branches, is required to adaptively manage stressors and may be diminished in depression (Harrison, 2002; Manji & Duman, 2001). Chronic stress has been associated with neuronal cell death or apoptosis (Uno et al., 1994). Stressors may impact neuroplasticity and survival through an interaction between 5-HT and brain derived neurotrophic factor (BDNF), which ensures 5-HT cell survival (Hayley et al., 2005). Cell death or reduced neurogenesis may lead to noticeable reductions in the volume of brain areas in postmortem studies of individuals with depression, specifically in the hippocampus (Kempermann & Kronenberg, 2003). It is currently unclear whether these neural morphological changes are a cause or result of depression.

*Regulation of gene expression.* Monoamines, which are seasonally variable, may impact gene expression, and may lead to neuron depletion in depression. It has become clear that changes in synaptic transmission affected by antidepressant medications initiate a biochemical cascade of reactions intracellularly that alters gene activity (Duman, Heninger, & Nestler, 1997; Shelton, 2000). Genes involved in neuronal survival and the stress response can be activated or inhibited (Thase et al., 2002), including CRH and BDNF which is involved in stress-induced cell death in the brain (Duman et al., 1997; Shelton, 2000). Antidepressants are thought to work by changing gene activity levels and preventing or reversing changes in the homeostasis of the HPA axis or cell survival processes. This role for antidepressants, given the seasonal variation in monoamine concentration, may be a link between seasons and depression in SAD.

*The role of sleep in depression.* Sleep is controlled by two processes, one of which is regulated by the circadian clock, and therefore may connect daily rhythms and depression in SAD. Individuals with depression often have diminished slow wave sleep (SWS) and early onset of the first period of rapid eye movement (REM) sleep, and depression is associated with an increase in the intensity of eye movements during REM sleep (Thase et al., 2002; Thase & Howland, 1995). Sleep may be a result of dysregulation of 5-HT transmission because neurons containing 5-HT inhibit the onset of REM sleep through the $5\text{-HT}_{1A}$ and $5\text{-HT}_2$ receptors (Thase et al., 2002). Many depressed individuals have normal sleep patterns, and some individuals sleep more than normal, perhaps in an effort to recover lost SWS earlier in the night (Thase, Fasiczka, Berman, Simons, & Reynolds, 1998). It is currently unclear whether sleep disruption is a cause or consequence of depression or depressive symptoms.

*The role of the circadian clock in depression.* A role for the circadian clock in depression is important to the present study of melanopsin in SAD. Sleep onset, cortisol levels, nocturnal growth hormone levels, melatonin, and core body temperature rhythms are all disregulated in some individuals with depression, and are controlled by the central clock (Wirz-Justice et al., 1995). It is likely that changes in activity in related brain areas and the levels of CRH and cortisol may affect the clock as a consequence of the neurobiological changes detailed above in depression (Thase et al., 2002). In addition, changes in the daily lives of individuals with depression such as changes in meal times, periods of social interaction, and exercise schedule may themselves cause changes in the function of the central clock (Ehlers, Frank, & Kupfer, 1988). Therefore, it is possible that dysregulation of

the circadian clock could be either a consequence or cause of depression symptoms. If it is a cause, then a role for melanopsin, the circadian photopigment, is supported.

*The specific role of monoamines in SAD.* As described above, 5-HT and NE have been implicated in non-seasonal depression as mediators of the stress response system, and are therefore of general interest in SAD as well. However, 5-HT is specifically implicated in SAD for a few reasons. First, brain serotonin levels are lowest in the winter season in healthy individuals (Lambert, Reid, Kaye, Jennings, & Esler, 2002; Sohn & Lam, 2005). Second, individuals with SAD treated to remission with light therapy relapse when levels of 5-HT and/or NE are experimentally reduced in neuroendocrine challenge studies (Lam et al., 1996; Neumeister et al., 1998), suggesting that light therapy may work through the monoamine signaling pathways. 5-HT modulates non-visual light input to the SCN, and 5-HT may mediate sleep disturbances due to a dysregulated circadian clock through postsynaptic G-protein signal transduction, an intracellular process initiated by neurotransmitter signaling (Sohn & Lam, 2005). Monoamines may act similarly in SAD as in non-seasonal depression as a vulnerability factor for stress sensitivity, with the difference being that this risk increases in magnitude during winter season. In addition, serotonin can inhibit the effects of light on the circadian clock through pathways independent of light input pathways, which may interact with the effects of non-visual light input in SAD (Yannielli & Harrington, 2004). Therefore, serotonin is important in SAD for two reasons: (1) it is implicated in non-seasonal depression, and (2) it varies in concentration across the seasons.

*Potential Mechanisms for a Biological Vulnerability to SAD*

The biological hypotheses of SAD reviewed above (i.e., photoperiodic, phase-shift, retinal subsensitivity, monoamine, stress response system) do not propose a specific

molecular mechanism, but are consistent with a hypothetical role for melanopsin in SAD. The genetic hypotheses of SAD, and those proposing a dysregulation of neurotransmitters do propose specific molecular mechanisms. Clinical findings reviewed above may explain the abnormal response to light observed in individuals with SAD. This abnormal response to light may be due to underlying problems in the light input pathway, the circadian clock, and/or neurotransmitter signaling pathways. Genes for molecular components of the light input pathway, the circadian clock, and neurotransmitter signaling pathways are possible candidates for a biological vulnerability to SAD and are reviewed below. Recent work has identified melanopsin as a non-visual photopigment, the first step in the light input pathway, making the gene for melanopsin a candidate gene for SAD. Previous research on the genetics of SAD has investigated genes that are molecular components of the circadian clock and neurotransmitter signaling pathways. To date, no studies have investigated the role of the gene for melanopsin in SAD.

*Previous Research on the Genetics of SAD*

*Heritability of SAD and seasonality.* Establishing the heritability of SAD and seasonality is critical before investigating candidate genes for SAD. An estimated 29-69% of the variance in seasonality (i.e., the tendency to change across the seasons in eating, sleep, weight, social activity, mood, and energy level) is believed to be heritable (Jang, Lam, Livesley, & Vernon, 1997; Madden, Heath, Rosenthal, & Martin, 1996). The larger of these two studies was conducted with 4639 adult twin pairs from a volunteer registry in Australia (Madden et al., 1996). The second study, using 339 twin pairs from Canada, found that seasonality was 69% heritable in men and only 45% heritable in women (Jang et al., 1997), suggesting that non-genetic factors account for more of the risk for SAD in women than men.

The heritability of SAD per se has not yet been estimated. Family studies estimate a 13-17% risk of SAD in first-degree relatives of SAD probands (Lam, Buchanan, & Remick, 1989; Thompson & Isaacs, 1988; Wirz-Justice et al., 1986), which is higher than the general population prevalence of 1.4-9.7%, depending on latitude in the U.S. (Rosen et al., 1990). To date, results from two independent twin studies and three independent family studies support a genetic basis for SAD, whereas one study (Sasaki, Sakamoto, Akaho, Nakajima, & Takahashi, 1998) found no familial association. The study by Sasaki et al. (1998) was conducted using 129 small Japanese families, whereas the other studies were conducted on primarily Caucasian populations that were Swiss-German, Canadian, Australian, and British. It is very possible that different ethnic or racial groups have differing degrees of genetic risk for SAD, and the above evidence suggests that, at least in predominately Caucasain populations of European origin, SAD is partly due to genetic factors.

*Genes known to be involved in non-seasonal depression.* Because SAD is Major Depressive Disorder with a seasonal pattern, genes that are known to be involved in non-seasonal depression are of interest to SAD research. These include serotonin, noradrenaline or norepinephrine, and dopamine genes (Sohn & Lam, 2005). A recent review of the genetics of depression found that all consistent associations between gene variants and depression involve the short allele of the serotonin transporter, 5-HTTLPR (Levinson, 2005). This variant has been associated with Major Depressive Disorder (MDD) diagnosis, suicidal behavior, bipolar disorder, and depression-related personality traits such as neuroticism as measured by the NEO Personality Inventory (Levinson, 2005). Caspi et al. (2003) found that 5-HTTLPR genotype did not predict depression scores or diagnosis, but did predict a relationship between negative life events and subsequent depression, suggesting that 5-

HTTLPR may be involved in stress reactivity as opposed to depression per se (Levinson, 2005). However, 5-HTTLPR is estimated to account for a very small but significant effect on depression diagnosis (Levinson, 2005). Tests of association between depression and other genes, including neurotransmitter-related genes, have not been replicated consistently (Levinson, 2005).

*The serotonin transporter 5-HTTLPR s allele and SAD.* The question of whether or not SAD has a genetic basis may be resolved by identifying specific genes associated with SAD. Previous candidate gene research has investigated the potential role of genes for components of neurotransmitter signaling pathways and the circadian clock in SAD because these biochemical systems have been theorized to be involved in SAD (Sher, Goldman, Ozaki, & Rosenthal, 1999). Because of the relative success of treating SAD with pharmacological agents that affect brain serotonin function (Moscovitch et al., 2004), and because of positive findings in MDD with the short allele of 5-HTTLPR, serotonin-related genes have been investigated in SAD.

*5-HTTLPR and SAD.* Similar to findings for MDD reviewed above, a polymorphism in the serotonin transporter gene (5-HTTLPR) has been associated with SAD and seasonality (Rosenthal et al., 1998). The sample of 97 SAD and 71 control individuals used in the Rosenthal et al. (1998) study are a subset of the sample proposed for use in the present study. Individuals were 88% Caucasian and were recruited in Bethesda, Maryland. The SAD sample included 74% unipolar, 1% Bipolar I, and 25% Bipolar II type SAD (Rosenthal et al., 1998). Individuals with SAD were more likely to have the short ('s') allele (45%) of 5-HTTLPR than controls (32%). The s allele accounted for only 7.4% of the variance in SAD in this sample (Rosenthal et al., 1998). Another study with a European Caucasian (Swedish,

Finnish, and German origin) sample of 82 SAD cases and 82 controls did not find any association between 5-HTTLPR and SAD diagnosis (Johansson et al., 2001). In a replication of that study, 147 new SAD cases and 115 new controls of Canadian, Swedish, Austrian, and Finnish ancestry were studied and no association between SAD and 5-HTTLPR was found (Johansson et al., 2003a). The same research group did find an effect of the s allele of 5-HTTLPR on degree of seasonality in a population-based (non-clinical) sample from northern Sweden selected for high and low seasonality (Johansson et al., 2003a). An interaction between 5-HTTLPR genotype and negative life events, such as the one found by Caspi et al. (2003) in MDD, has not yet been tested in SAD. It is possible that the only positive association found for 5-HTTLPR and SAD in the American sample (Rosenthal et al., 1998) is a false-positive result, or a result that only holds for American Caucasian populations. This finding has not been replicated, and no findings for 5-HTTLPR account for all of the variance in SAD and/or seasonality, leaving open a role for other genetic risk factors.

*The serotonin receptor 5-$HT_{2A}$ is involved in SAD but not seasonality.* Enoch et al. (1997) identified and associated a polymorphism in 5-$HT_{2A}$ (-1438G/A) with SAD but not with seasonality as measured by the global seasonality score (GSS) of the Seasonal Pattern Assessment Questionnaire (SPAQ; Rosenthal, Bradt, & Wehr, 1984a). The study conducted by Enoch et al. (1999) used 67 SAD and 69 control samples that are a subset of the larger sample proposed for use in the present study, a sample that is predominately Caucasian and was recruited in Bethesda, Maryland. The samples used by Rosenthal et al. (1999) and Enoch et al. (1999) are the same sample, and only differ in sample size due to some missing samples that were not successfully genotyped for each study. Individuals with SAD were more likely to have the -1438A variant (47%) than controls with no history of depression and

minimal seasonality (36%; Enoch et al., 1999). The -1482G/A polymorphism in 5-$HT_{2A}$ and the 5-HTTLPR s allele were not associated with seasonality (GSS) in this sample, and the authors suggested that -1438A variant in 5-$HT_{2A}$ may be associated with depressive symptoms in SAD patients, but not with seasonality as indexed by GSS (Enoch et al., 1999). This association between 5-$HT_{2A}$ and SAD has been tested in other samples. The 102-T/C polymorphism in 5-$HT_{2A}$ was more common in individuals with MDD with a seasonal pattern (i.e., SAD) than in those with non-seasonal MDD in a sample of 159 patients with MDD and 164 unrelated, healthy controls conducted in Barcelona, Spain (Arias, Gutierrez, Pintor, Gasto, & Fananas, 2001).

In the study by Arias et al. (2001), no association between 5-$HT_{2A}$ and cases of MDD (with or without seasonal pattern) and controls was found, suggesting that 5-$HT_{2A}$ may be a risk factor specific to SAD and not to MDD without a seasonal pattern. However, this directly contradicts the findings of Enoch et al. (1999). In addition, Johansson et al. (2001) found no association between 5-$HT_{2A}$ and SAD or seasonality in a northern European Caucasian population, and Ozaki et al. (1996) found no association of 5-$HT_{2A}$ in a comparison of 50 individuals with SAD from Bethesda, Maryland (a sub-set of the sample used in the present study), and controls from two sources, 70 Centre d'Etude du Polymorphism Humain (CEPH) population controls, and 62 normal controls recruited in Bethesda, Maryland (a sub-set of the samples used in the present study). Due to the inability to consistently replicate the associations between the s allele of 5-HTTLPR and 5-$HT_{2A}$ with SAD and/or seasonality, it is possible that the initial positive reports were false-positives. Other studies of serotonin receptor gene polymorphisms (5-$HT_{1A,1B,1E,1D}$ and 5-$HT_{2A,2C}$) have

not identified any polymorphisms associated with SAD (Ozaki et al., 1996; Sher et al., 1999), some of which utilized the samples proposed for use in the present study.

*Dopamine-related genes and SAD.* According to the monoamine hypothesis reviewed above for depression, dopamine-related genes are candidate genes for SAD. The neurotransmitter dopamine is associated with eating behavior, activity levels, and weight gain (Levitan et al., 2004a). Individuals with SAD report seasonal changes in eating, activity, and weight gain, suggesting possible involvement of dopamine signaling pathways in SAD. Levitan et al. (2004) found that the seven repeat allele of the Dopamine-4 Receptor gene (DRD4) was associated with obesity and binge eating in a group of women with SAD (Levitan et al., 2004a; Levitan et al., 2004b). However, it is unknown if the seven repeat allele of DRD4 is more common in individuals with SAD compared to controls or if this DRD4 variation is associated with seasonality, in general.

*Clock genes and SAD.* Individuals with SAD may have dysregulated circadian rhytms as reflected by phase delays in core body temperature, dim light melatonin onset, and a preference for evening over morning (diurnal preference), suggesting possible involvement of circadian clock genes in SAD. Genes for the molecular basis of the circadian clock (CLOCK, Period2, Period3, and NPAS2, *Timeless* and *Casein kinase 1-epsilon*) were studied in four groups of European individuals, SAD ($n$ = 159), matched controls ($n$ = 159), and high seasonality ($n$ = 127) and low seasonality ($n$ = 98) populational samples from northern Sweden (Johansson et al., 2003b). The 471 Leu/Ser polymorphism in NPAS2 was associated with SAD but not with seasonality, and none of the other genetic variants in the other genes were associated with either SAD or seasonality (Johansson et al., 2003b). Individuals with SAD were more likely to have the Leu/Leu homozygous assumed recessive genotype (6%)

than controls (1%). Although this is a positive finding, only 6% of individuals with SAD had the Leu/Leu genotype, and, therefore, the NPAS2 polymorphism does not account for a large percentage of variance in SAD. This association between SAD and NPAS2 has not been replicated. Taken as a whole, previously identified genetic factors for SAD do not account for more than 15% of the estimated 29% to 69% of variance in SAD status attributed to genetics, underscoring the need for more studies on the genetic factors involved in SAD.

*Molecular components of the light input pathway are candidate genes for SAD.* Proper entrainment of the central clock to environmental time requires input regarding environmental light levels, which is provided by way of the light input pathway. Therefore, variations in genes for the molecular components of the light input pathway are also candidates for SAD. Photoreception is the first step in the light input pathway, and recent research suggests that both visual and non-visual photoreceptors contribute to light information projected centrally in trichromatic primates (Dacey et al., 2005). Preliminary analyses on one of three previously identified variants in the gene for melanopsin and SAD (P10L) does not suggest an association between P10L and SAD, but this is not a conclusive test of association between SAD and the entire melanopsin gene because only one variant is tested. These preliminary data are presented below in the Preliminary Data section. The two additional mutations (I394T and D444G) in the gene for melanopsin have yet to be studied in SAD, and it's possible that there may be other, as of yet unidentified loci that are important in SAD. In addition, a larger sample and a better control group may reveal an association between P10L and SAD that was not found in the Preliminary study.

*Gene-environment interactions proposed for SAD.* Genes that are light-dependent interact with environmental factors such as light intensity and timing, and are candidate

genes for SAD. Below, the involvement of other environmental factors such as stress in SAD are reviewed. Genes, including those mentioned above involved in transmitting non-visual light input, can be expected to interact with environmental factors that affect light levels. Although latitude and day of year are the two factors that determine day length, many other factors can affect how much bright light an individual is exposed to, including moving from a brighter to a darker home or office, having windows that are coated to reduce light transmission for energy conservation, living in a deep valley in a mountainous region, cloudy or foggy weather, or developing cataracts or other visual impairments (Rosenthal, 2006). Therefore, the climate an individual lives in may interact with any genetic factors present, in the ultimate expression of SAD.

Researchers suggest that another environmental factor that could interact with genes is life stress (Rosenthal, 2006). The role of serotonin genes in stress sensitivity is described above. The occurrence of work stress, a new job, family stressors, or moving to a new location (i.e., examples of stressful life events) may interact with serotonin genes and other vulnerability factors in SAD raising the risk of developing an episode of major depression in winter (Rosenthal, 2006). In addition, life stressors such as the loss of a member of the social support network may lead to changes in social rhythms such as changes in the timing and amount of social contact.

Social activity is a powerful cue in humans with the capacity to entrain the biological clock, and is termed a social zeitgeber, or 'time giver' (Ehlers et al., 1988). Researchers suggest that a disruption of social rhythms, which may result in changes in circadian rhythms, could be responsible for triggering the onset of a major depressive episode in vulnerable individuals (Ehlers et al., 1988). Manipulations that increase arousal and can

affect the clock in mammals include novelty-induced activity, social interaction, saline injections, and handling (Yannielli & Harrington, 2004). It is plausible that the environmental factor of changing social zeitgebers may interact with genes involved in the clock and signaling pathways for the clock, as well as genes related to stress sensitivity. Some data suggest that sensitivity to social zeitgebers correlates with seasonality in individuals with SAD, suggesting that individuals with SAD are more responsive to social rhythms than less-seasonal individuals (Reid, Towell, & Golding, 2000). Additionally, if social cues are more important for entrainment in individuals with decrements in the function of the light input pathway, the loss of social zeitgebers through life events could interact to confer even greater risk of developing a depressive episode. However, data shows that light is the most important entraining cue in humans.

*Personality, depression, and genetics.* The interaction between genes, personality, and depression is important given the present study and it's focus on genetic factors in SAD. Research shows that depressive personality is closely etiologically related to Axis I depressive disorders, particularly chronic forms of depression (Klein, Durbin, Shankman, & Santiago, 2002). As SAD is, by definition, a chronic, recurrent form of depression, investigating personality factors that may be associated with SAD is indicated. A review of the personality dimensions that are proposed to be risk factors for MDD and SAD follows, with data on the interrelationship of genetic factors presented when available.

*Neuroticism and Openness in MDD and SAD.* MDD is associated with high levels of neuroticism (N) and low levels of extroversion (E; Klein et al., 2002), although high N is found in most forms of psychopathology (i.e., anxiety disorders, substance dependence, and antisocial personality disorder) and is not specific to MDD (Clark, Watson, & Mineka, 1994;

Krueger, 1999). N may be partly a function of depressive state, and data suggest that individuals high in N may generate more stressful events that interact with personality and precipitate depressive episodes (Kendler et al., 2003; Van Os & Jones, 1999).

*Serotonin genes and neuroticism in depression.* Researchers have consistently identified an association between a polymorphism in the serotonin transporter, 5-HTTLPR, and N (Greenberg et al., 2000). A meta-analysis of the association between 5-HTTLPR and trait anxiety found that 5-HTTLPR has a small but reliable influence on N, but did not find a reliable association between this gene and anxiety behavior in the absence of the moderating effect of N (Schinka, Busch, & Robichaux-Keene, 2004). Schinka et al. (2004) and other researchers suggest that research will be maximized by the use of personality measures to identify moderating effects of personality on relationships between stressful life events, genes, and behavior (Van Os & Jones, 1999). Using a twin study design, a large association between seasonality as measured by the GSS and N was found to have a common genetic basis as opposed to an environmental basis (Jang, Lam, Harris, Vernon, & Livesley, 1998). In a study comparing 5-HTTLPR, seasonality, and N, researchers found that the effect of 5-HTTLPR on seasonality was largely independent of the effect of 5-HTTLPR on N, but that the gene had a larger effect on the covariation between N and seasonality than it did on either trait alone (Sher et al., 2000). In a sample of individuals with SAD, this test was repeated and results showed that seasonality and N were independent constructs, but both are predicted by 5-HTTLPR genotype (i.e., pleitropy; Gordon, Keel, Hardin, & Rosenthal, 1999). Therefore, the serotonin transporter interacts with neuroticism and seasonality in the

expression of SAD. Similar complex interactions would be expected of an association between melanopsin in SAD.

*Openness and SAD.* A distinction between individuals with SAD and those with non-seasonal depression is that individuals with SAD score higher on the Openness (O) scale of the NEO-Five Factor Inventory (Costa & McCrae, 1992), and may score lower on N than individuals with non-seasonal depression (Jain, Blais, Otto, Hirshfeld, & Sachs, 1999). Specifically, on the O scale, individuals with SAD scored higher than those with non-seasonal depression on the Aesthetics and Feelings sub-scales (Bagby, Schuller, Levitt, Joffe, & Harkness, 1996). The authors propose that this finding suggests that individuals with SAD are sensitive to the environment and to changes in light (i.e., the Aesthetic sub-scale), and vulnerable to amplification of the normal dysphoria associated with winter (i.e., the Feeling sub-scale; Bagby et al., 1996). Genes that might interact with these personality traits are hypothesized to be those involved in non-visual processing of environmental stimuli, and include melanopsin as well as other genes involved in processing visual and non-visual environmental light input. It is possible that melanopsin mediates the association between SAD and a personality disposition to be sensitive to environmental conditions because melanopsin is involved in non-visual light input.

*Harm avoidance and low self-directedness in depression.* MDD is also associated with high harm avoidance and low self-directedness, and cooperativeness as assessed in Cloninger's personality dimension model (Cloninger, Bayon, & Svrakic, 1998a; Joffe, Bagby, Levitt, Regan, & Parker, 1993; Richter, Eisemann, & Richter, 2000), although this finding is not specific to depression (Ampollini et al., 1999). In anxiety, genetic

contributions of multiple genes have been attributed to the trait of harm avoidance (Cloninger et al., 1998b). Associations between 5-HTTLPR and harm avoidance in non-clinical samples have demonstrated mixed results (Greenberg et al., 2000). In non-seasonal depression, 5-HTTLPR is associated with low novelty-seeking scores, the Dopamine Receptor D4 (DRD4) is associated with low harm avoidance, and a polymorphism in a Mono-amine Oxidase related gene, MAO-A, is associated with decreased persistence scores (Serretti et al., 2005). Although the results for traits in Cloninger's dimensions of personality as not as clear as for neuroticism and openness, the data do suggest that the contribution of serotonergic and dopaminergic genes to mood disorder risk is mediated by personality traits (Serretti et al., 2005).

*Harm avoidance in SAD.* Individuals with winter SAD demonstrate high harm avoidance, at rates equivalent to individuals with non-seasonal depression (Maeno et al., 2005). Individuals with SAD have higher scores in harm avoidance as well as lower scores in novelty seeking, self-directedness and cooperativeness when compared to controls (Thierry et al., 2004). 5-HTTLPR is only associated with lower self-directedness scores in SAD patients (Thierry et al., 2004). It is possible that the contribution of neurotransmitter-related genes in

SAD is mediated by personality traits in a manner similar to that demonstrated in non-seasonal depression. The data on genes, personality, and SAD and non-seasonal depression reviewed above support the inclusion of measures of personality in studies of genetic risk factors for depression because personality factors may mediate the relationship between genes and behavior. In addition, it would be interesting to test whether or not the

above associations between high neuroticism and high harm avoidance can be replicated in the present study.

### *Candidate Gene Association Studies*

The present study tests an association between the melanopsin gene and SAD or seasonality in a sample of individuals with SAD (cases) and healthy controls. Multiple study designs have been employed in research to test for gene-disorder associations, and there has been debate in the field about study methodological and design issues in testing gene-disorder associations. These study methodology and design issues and the components being debated by researchers are reviewed below in order to provide a background for discussing details of the present study design and methodology. Alternate study designs are described to provide a rationale for the present study design.

#### *Introduction to Gene-Disorder Association Testing*

The range of methods for gene-disorder association testing is presented to develop a rationale for the present study methodology. Multiple approaches to testing gene-disorder associations exist, ranging from testing a single candidate gene for an association with a disorder of interest to genome scans in which the entire genome is tested for an association. The benefits and disadvantages of these differing approaches follows in order to demonstrate the rationale behind the study design proposed for the present study. The proposed study will test a hypothesized role for a candidate gene in SAD as opposed to an exploratory, hypothesis-generating approach in which all genetic material is tested for an association. Limiting the proposed study to the gene for melanopsin precludes discovery of other genes that may be part of a polygenic mechanism (Hoh, Wille, & Ott, 2001), which is likely in the etiology of SAD. In addition, examining candidate genes for SAD other than melanopsin is

outside of the scope of this project. Methods to manage disadvantages of candidate gene case-control studies including statistically maximizing power, minimizing Type I errors, and controlling bias in a case-control sample are reviewed below.

A discussion of the research methodology behind identifying important genetic sequence variation is provided as a foundation for understanding the research methodology proposed for the present study. A large source of sequence variations in the genome present in a given population occurs as single nucleotide polymorphisms (SNPs). SNPs are variations in the genome occurring approximately every 200-300 base pairs (Lee, Choi, Lee, & Lee, 2005) that may or may not be of biological significance. SNPs are biallelic, with only two possible sequence variations, lending to their detection by simple, high-throughput methods such as TaqMan assays (described below) to generate fast, accurate genotypes (Lee et al., 2005).

*Disadvantages of Case-Control Association Studies*

The main methodological problem with association studies of the type proposed in the present study proposal is non-replication. Multiple explanations for non-replication of association studies have been suggested. For example, non-replication of single-SNP association studies may occur when *in vivo* function of a protein depends on the interaction of combinations of SNPs (Clark, 2004; Wang & Zhao, 2003). Testing for epistasis, or the interaction of melanopsin variations with other genes, is beyond the scope of this project. The present study employs a combined functional SNP (fSNP) - haplotype analysis of the melanopsin gene. This means that 3 specific loci (fSNPs) are tested for an association with SAD, and in addition, the regions between these loci and immediately before and after the

melanopsin gene are tested (haplotype analysis), a strategy designed to capture all potentially important sequence variation.

*Advantages of Population-Based Case-Control Samples*

Gene-disorder associations are tested in either family-based (including twin studies) or population-based case-control designs using unrelated individuals. Population stratification is the presence of subgroups in a population, such as different racial groups, which may differ in characteristic ways at certain genetic loci (Pritchard & Donnelly, 2001), and is commonly cited as a disadvantage of population-based designs. Family-based designs compare cases to unaffected relatives. Family designs are unlikely to be biased by population stratification because controls and cases are necessarily drawn from the same population (i.e., because they are from the same family; Thomson, 1995). Disadvantages of family-based association studies include the expense and time required to recruit and genotype family members and lower statistical power than population-based studies for a given sample size (Risch & Teng, 1998). Methods for managing the potential effect of population stratification on the present study are discussed below.

Population-based case-control study designs comparing cases to unrelated, unaffected controls are commonly available as these are samples collected for traditional psychopathology studies (Hutchison, Stallings, McGeary, & Bryan, 2004). These population-based case-control samples are often sufficiently sized to detect associations with power comparable or superior to that in family-based designs (Long & Langley, 1999; Morton & Collins, 1998; Risch & Teng, 1998; Schaid & Rowland, 1998). Genes with relative risks as low as 1.5 may be studied in sufficiently powered association tests using reasonable sample sizes in case-control designs (Risch, 2000).

*Haplotype Structure*

The extent to which variations within in a gene are correlated or interactive must be understood to prevent study design flaws that may contribute to non-replication (Tiret et al., 2002). Methods for characterizing the degree of correlation between variations in a gene are reviewed below to provide background for the study methodology in the present study proposal. A haplotype is a particular combination of closely linked genetic loci on a chromosome which tend to be inherited together and are not easily separable by recombination (Gelehrter, Collins, & Ginsburg, 1998). Recombination is the process in which gene fragments are exchanged between parental alleles leading to new genetic sequences. Haplotypes are divided where DNA is broken and rejoined with a new sequence through recombination (Gelehrter et al., 1998). Determining the haplotype structure of a candidate gene region can improve association analyses so that important sites of variation (unknown variants) are identified and redundant variations are removed from the analysis (Clark, 2004). Genetic variation can arise in two relevant ways: (1) through point mutations in which one nucleotide is changed to another and (2) through recombination in which a chromosomal segment is exchanged for another such that the resulting allele resembles part of both of the two ancestral alleles (Clark, 2004).

*Linkage Disequilibrium: A Measure of Correlation Between Loci*

Linkage disequilibrium (LD), the nonrandom association between variants at different polymorphic sites (Weir, 1996), is the principle behind identifying haplotype blocks. Two polymorphisms at loci that are close together on the same chromosome are linked if they are inherited together at a rate greater than the 50% expected for unlinked genes (Gelehrter et al., 1998). The present study will identify regions of high LD, using measures of $D' \geq 0.9$

(described below) to define haplotype "blocks" of DNA that show few structural changes within populations, and then perform tests of association between identified haplotypes and case status (SAD vs. control). Haplotype blocks are composed of regions with strong inter-marker LD (Twells et al., 2003), and are flanked by hotspots that have recombination rates of between 10 and 1,000 times the rate for proximal regions (Clark, 2004; Crawford et al., 2004). Despite the general tendency for LD to increase with decreasing distance between loci, rates of LD are not uniformly distributed across the genome (Tiret et al., 2002). This is why the entire gene for melanopsin as well as regions surrounding the gene will be tested to see if they are in LD with other regions of the gene. One variant may have unexpectedly low rates of LD with other variants in a gene despite being relatively close if a hotspot of recombination is near (Tiret et al., 2002; Twells et al., 2003). Association studies using SNPs are based on the assumption that a SNP tested for association with a disorder is either functionally relevant to the disorder itself, or is in LD with the unidentified, putative disease-risk mutation (Risch, 2000). Therefore, determining the haplotype structure of a gene is important to inform tests of association and will be one strategy employed in the present study.

*Haplotype structure and statistical treatments to maximize power.* Analyzing the haplotype structure of a gene can have two significant impacts on the power of a test of association; (1) the degrees of freedom can be appropriately controlled, and (2) the adjustment for multiple testing can be minimized appropriately. These are introduced below to inform the reader of these issues with respect to the study methodology being proposed for the present study.

*Haplotype structure and false-positive or Type I errors.* The false-positive rate in case-control studies has been attributed to a failure to take into account haplotype structure and uneven rates of recombination within genes (Tiret et al., 2002). There are two reasons why a failure to take haplotype structure into account is potentially problematic; (1) one polymorphism may not account for all of the variation that is important in a gene, and (2) two polymorphic sites may be correlated if they reside within the same haplotype, producing information about one of the variants that is redundant (Clark, 2004). When two polymorphisms that are inherited together are treated individually, the degrees of freedom of a statistical test are increased unnecessarily (Clark, 2004). If polymorphisms are analyzed individually, even if only three polymorphisms in a gene are considered, the possible combinations of genotypes is $3^3 = 27$, yielding 27 possible outcome categories to analyze. The degrees of freedom for such a test would be $(n - 1)$ or 26. The degrees of freedom for haplotype analysis are limited to the number of observed haplotypes.

*Bonferroni corrections to control the Type I error rate.* If each polymorphism in a candidate gene were tested individually and is inherited independently, Bonferroni correction of the alpha level would be advisable to control for multiple tests of association (Lander & Botstein, 1989). However, if the multiple polymorphisms are correlated, Bonferroni correction may be overly stringent, leading to an increased rate of false-negatives (Newton-Cheh & Hirschhorn, 2005). Bonferroni correction would be overly-stringent in cases involving multiple polymorphic sites on a single haplotype block, and failure to use a Bonferroni correction would be a mistake in cases involving polymorphisms that are not in complete LD and span multiple haplotype regions (Cheverud, 2001). Determining how many haplotypes exist in a candidate gene and considering each haplotype as one hereditary

event is the most parsimonious method of analysis, maintaining the power of the test to detect an association by limiting the degrees of freedom while adhering to an accepted standard of correction for Type 1 error when conducting multiple tests (Tiret et al., 2002).

*Non-Replication of Case-Control Associations*

As mentioned above, population-based case-control association study findings are often not replicated, suggesting a high rate of false-positive results (Risch, 2000). Recently, a meta-analysis of population-based case-control association studies of complex diseases found that out of 166 associations that were tested at least 3 times, only 6 were consistently replicated, and only half were replicated one or more times (Hirschhorn, Lohmueller, Byrne, & Hirschhorn, 2002). A commonly cited reason for non-replication is that population stratification in one study may have led to spurious results that would not be repeated in studies with more homogenous samples. In addition to the sources of bias common to all case-control study designs, candidate gene studies are vulnerable to bias that is unique to genetic studies such as population stratification, further reviewed below.

*Factors contributing to Type I error.* Some researchers suggest that the high false-positive rate seen in population-based case-control studies is more likely to be due to factors other than population stratification including a weak rationale for the involvement of the candidate gene (Risch, 2000), poor study design or low power (Wacholder, Chanock, Garcia-Closas, El Ghormli, & Rothman, 2004), genotyping errors (Hoh et al., 2001), and a publication bias in favor of significant results (Pritchard & Donnelly, 2001). A recent review of association studies published in three volumes of the Journal of Human Genetics in 1997 and 1998 found that published reports are not different from results obtained through chance alone (Terwilliger & Weiss, 1998). This study by Terwilliger and Weiss (1998) assessed

studies on genes for a variety of disorders. A strong rationale for the involvement of a given candidate gene is critical prior to association testing to minimize the potential of a false-positive result. Given the potential role of melanopsin in SAD, this study has a plausible rationale for the specific involvement of the gene for melanopsin. Genotyping errors are minimized through the laboratory methods described below. Because non-replicated studies often report effect sizes that are higher than those in subsequent studies, power is estimated for one analysis proposed for the present study (below) to ensure that this study is sufficiently powered to identify magnitude of effect we hypothesize for the present study (Ioannidis, Ntzani, Trikalinos, & Contopoulous-ioannidis, 2001).

*Bias in Case-Control Studies*

*Bias due to differences between cases and controls.* Some study design issues are not unique to genetic studies, but are common to all case-control study designs, including the choice of a suitable control group (Cordell & Clayton, 2005). Case-control studies rely on the assumption that comparison groups have the same chance for exposure to risk factors for a disorder. Therefore, efforts to ensure that groups do not differ in risk exposure are critical. For example, individuals of different socio-economic status (SES) may have different exposures to risk factors for the disorder of interest due to increased stressors, chronic strain, and economic disadvantage (Daly, 2003). Matching case and control groups on age, gender, and race ensures that groups do not differ on these demographic characteristics. Cases in a given study may also differ from controls on the basis of 'patient-hood' as they self-identified as having a particular disorder and/or sought treatment. In the case of SAD, ideal controls would not have any Axis I disorder and have no family history of Axis I disorders. The present study proposes using genetic samples collected from people with SAD, and no

other Axis I disorders, who presented for treatment at an NIH clinic, and controls with no personal or family history of Axis I disorders from an NIH healthy volunteer bank, suggesting that the groups may differ in 'patient-hood' and diagnosis, but not in their willingness to participate in research. Further details on the inclusion and exclusion criteria for the present study are presented below in the *Methods* section, as well as issues regarding how similar cases and controls are in the present study.

*Significance testing, outliers, and study power.* Some issues of design that may bias the results of case-control studies are not unique to genetic studies, but are addressed in a special manner in genetic studies (Schork et al., 2001). These include assessing statistical significance, handling genetic outliers, and generating power estimates for group comparisons (Schork et al., 2001). Correcting the alpha-level for multiple tests may be overly punitive when two tests of SNPs that are inherited together are treated as discrete heritable events as opposed to one, as described above. Outliers may be individuals with risk-conferring polymorphisms for a disorder that differ from those in the rest of the sample. Criteria developed for assessing which outliers should be removed in the present study are described below. Various threats to study power are discussed in this proposal and a power estimate is provided below. Post-hoc power assessments will be provided, pending genotype frequency information for the melanopsin gene in the study sample.

*Population stratification, haplotypes, admixture, pleiotropy, and functional significance.* Further issues of study design are specific to genetic studies. These include population stratification and genetic background, haplotype analysis, admixture, pleiotropy, and physiological significance (Schork et al., 2001). Each of these issues can lead to questions as to whether an identified association is a causal or spurious association.

*Population stratification and Type II errors.* Population stratification, or genetic differentiation between groups, has been cited as a source of false-positive (Type I error) as well as false-negative results (Type II error; Khlat, Cazes, Genin, & Guiguet, 2004). The effect of differing genetic backgrounds reflecting different populations-of-origin is discussed in detail below and methods are proposed to control for population stratification in the event of positive findings. Controlling for population stratification is not performed in the present study because results showed no association between melanopsin and SAD or seasonality. Despite the theoretical possibility that false-negative results are due to stratification, genomic controls for stratification are only considered worth their cost and time in the event of a demonstrated association because the risk of obtaining false negative results, evaluated using simulation studies, is estimated to be low (Gorroochurn, Hodge, Heiman, & Greenberg, 2004). In the event of negative findings, the variability of allele frequencies among different demographic groups (i.e., women only, Caucasian only) can be analyzed to see if the frequency of genetic markers varies across potential subpopulations (Dikeos et al., 2006). It is believed that genetic markers that vary in frequency across populations usually do not have a biological impact on the variation of the risk of common diseases (Ioannidis, 2007). Therefore, because the threat of population stratification to Type II errors is low, and statistical methods are available to identify any such effect, genomic control will not be used in the present study.

*Haplotype diversity.* Failure to take haplotype diversity and heterogeneity into account can lead to both positive and negative spurious results. Accordingly, statistical tests that allow for the possibility that multiple haplotypes will show differential frequency in the comparison groups are proposed. Pleiotropy is a situation in which subphenotypes of a larger

class of disorders are related to distinct genetic risk factors, but gene-disorder associations are not apparent because all subphenotypes are analyzed together. Post-hoc testing in this study will seek to identify any symptom presentations that segregate with specific haplotypes or fSNPs to determine if the current data set provides any support for the existence of subphenotypes in SAD. The above study design issues are reviewed to provide background for discussing the specific methodological issues in the present study proposal.

*Population Stratification as an Issue in Genetic Research*

Case-control studies are vulnerable to population stratification. Populations are structured or stratified when the comparison groups (i.e., the cases with the disorder and controls without the disorder) differ by age, race, gender, SES, or another factor that increases or decreases both risk of the disorder and allele frequency (Risch, 2000). For population stratification to bias the conclusion of a study, there must be both a difference in allele frequency and disease rate between comparison groups (Thomas & Witte, 2002). That is, any variation in allele frequency must be correlated with variation in disease rates between subpopulations. As Pritchard and Rosenberg (1999) explain, when a disorder is more common in one subgroup, the disorder will also be associated with any genetic markers that are more frequently represented in that subgroup. If this subgroup is one strata in a larger population of cases in a case-control analysis, the markers associated with the subgroup might appear to be related to case status when the markers are in fact not related to case status within the population as a whole (Gorroochurn et al., 2004). The necessity of controlling for population stratification as a potential source of bias in population-based case-control samples is a topic of debate (Hutchison et al., 2004). Population structure has recently been shown to cause false-positive results in simulations of case-control association

studies (Campbell et al., 2005; Khlat et al., 2004; Koller, Peacock, Lai, Foroud, & Econs, 2004).

*Population stratification as a potential confounder in SAD studies.* The present study includes case and control samples that are 92% and 96% Caucasian, 4% and 2% African American, 3% and 2% Hispanic or Latino, and 1% and 0% Asian American, respectively. If rates of SAD do not vary across different demographic groups or between subgroups within demographic groups, then confounding due to population stratification cannot be present (Thomas & Witte, 2002). However, SAD incidence does vary by gender and age, and may vary by race at the latitude where samples for the present study were collected. For population stratification to have a confounding effect, any variation in allele frequency must be correlated with variation in disease rates between subpopulations (Thomas & Witte, 2002). SAD is approximately 1.8 times more common in women than men, and the risk of SAD appears to be greatest in the 20s and to decrease between mid-life and old age (Magnusson & Boivin, 2003). Although geographic gradients in SAD prevalence are likely, these region-specific differences in SAD incidence are likely to be explained by latitude and climatic and atmospheric variables (e.g., global radiation, cloud cover) than by location per se (Risch, Burchard, Ziv, & Tang, 2002).

*Potential for stratification in the present sample.* The general population rate of SAD in Maryland, where the current study samples were collected, has been estimated to be between 4.3 and 6.3% (Kasper, Wehr, Bartko, Gaist, & Rosenthal, 1989; Rosen et al., 1990). One study reported a 5.4% prevalence rate for SAD among African American college students in the greater Washington, D.C. area (Agumadu et al., 2004), which is comparable to rates reported for other racial groups in the same region. This suggests that there may be

no differences between Caucasians and African Americans in SAD incidence in the region where this study was conducted. However, as variation in rates of SAD between all groups included in this study (i.e., European Americans, African Americans, Hispanic Americans, and Asian Americans) cannot be ruled out, population stratification based on race remains a possible confounder. In addition, variations in SAD prevalence based on SES are possible, but this was not measured in the present study. Most studies have found that recent immigrants to northern latitudes have a higher prevalence of SAD than native-born individuals (Magnusson & Boivin, 2003). Length of residence in the study area and latitude of birth were not recorded for individuals whose samples are being proposed for use in the present study, although this would be helpful information to have in future studies.

*Types of population stratification.* Although it is clear that race cannot be defined on the basis of genes alone, the single most important factor contributing to genetic differentiation in human populations is race as defined by geographical ancestry (Risch et al., 2002). Most variation in human populations is within groups (approximately 75% of total variance) with a smaller amount of variation (5%-15% of variance) observed between major continental groups (Shriver et al., 2004). However, two individuals from the same population (i.e., two Caucasians) tend to be more genetically similar than two individuals from different populations (i.e., a Caucasian and an Asian; Risch et al., 2002). In addition, a small fraction of genetic loci explains a large proportion of the variance between populations (Shriver et al., 2004). As differential allele frequency or disorder prevalence between groups can lead to spurious associations, research demonstrating genetic differentiation and disease risk differentiation between races underscores the importance of considering race in gene-disorder association testing (Risch et al., 2002).

*Ethnic heritage versus geographical stratification.* There are two ways in which populations may be structured on the basis of race, ethnic heritage or major continental groupings. Two or more subpopulations may exist, or the population may be admixed, meaning it is characterized by the mixture of two ancestral populations (Chen, Zhu, Zhao, & Zhang, 2003). In the case of admixture, a single individual may have a fraction of alleles inherited from one ancestral population, and a complementary fraction of alleles inherited from another ancestral population (Chen et al., 2003). Admixture is thought to result from intermarriage between individuals from different groups such that the next generation has allele frequencies that reflect both parent populations in different areas of the genome (Cardon & Bell, 2001). Admixture is likely to be present in our sample, as is the case in other American samples.

*Population structure and admixture.* Both subgroups and admixture can lead to spurious gene-disorder associations if the risk of disease or allele frequency depends on degree of admixture or group membership (Pritchard & Donnelly, 2001). Populations of self-identified African American and Caucasian individuals may be genetically admixed, even if individuals self-identify as belonging to only one group. In our predominately Caucasian sample (92% of SAD cases, 96% of Controls), stratification within the Caucasian group cannot be ruled out as potential confounding effect because the cases and controls could theoretically contain different rates of admixture or fractions of ancestry from multiple ancestral parent populations (Pritchard & Rosenberg, 1999). Because it is possible that the population in the present study contains discrete subgroups as well as admixture, the optimal statistical correction for population stratification would be robust to both types of population stratification.

*Self-reported race compared to genetically estimated admixture.* An individual's self-defined race may be correlated with genetically estimated population membership and with cultural and environmental factors that could impact disease risk (Thomas & Witte, 2002). However, it is unlikely that culturally defined ethnicity completely reflects genetic differentiation, making self-reported race an incomplete measure of population stratification (Risch et al., 2002). Estimates of Caucasian admixture in African Americans range from 12% to 23% based on regional differences (Risch et al., 2002). However, in some cases, self-reported ancestry is equally or more strongly correlated with disease status than genetically estimated admixture (Risch et al., 2002; Sinha, Larkin, Elston, & Redline, 2006; Williams, Long, Hanson, Sievers, & Knowler, 2000). In the present study, grandparent ancestry is unavailable, but self-reported race was collected. It is evident that self-reported race, in the absence of ancestral background, does not completely capture admixture or the existence of subpopulations in the present sample, making the use of genomic control markers an important, independent test in the event of significant associations (Risch et al., 2002). Self-reported race and genetic differentiation are considered two different surrogate measures that may reflect latent strata associated with allele frequency, however, only self-reported race is available for the present study sample.

*Population structure in Caucasian American samples.* North American Caucasian populations are more heterogeneous than, for example, Finnish Caucasian populations (Risch, 2000), but are more homogenous than African American populations. In most case-control studies using U.S. Caucasians and European Americans, evidence for population structure has been weak or absent (Ardlie, Lunetta, & Seielstad, 2002; Pankow, Province, Hunt, & Arnett, 2002). Population stratification has been identified in some studies using

likely structured populations (i.e., African American, African Caribbean, Native American and Hispanic American) through the use of genomic control loci (Gorroochurn et al., 2004; Hoggart et al., 2003; Kittles et al., 2002; Knowler, Williams, Pettitt, & Steinberg, 1988; Schork et al., 2001). Although whites of European or Middle Eastern origin are generally considered homogenous for the purposes of candidate gene studies (Thomas & Witte, 2002), stratification was recently identified in a sample of European Americans (Shriver et al., 2005). In the publication "The Evaluation of Forensic DNA Evidence," The National Research Council recently adopted the position that the effect of stratification is likely to be negligible within an ethnic group such as Caucasians (Curran, Buckleton, & Triggs, 2003; National Research Council and C.O.D.F. Science, 1996). Since the present sample is largely Caucasian, this finding has a direct bearing on the potential for stratification in the present study.

*Ethnic variation within Caucasians in Maryland.* In some parts of the U.S., ethnic variation within Caucasians may be less than in other areas, requiring more sensitive measures of stratification. For example, 80% of Caucasians in Illinois reported their ancestry to be German (Thomas & Witte, 2002). Residents of Maryland, the area in which the present study sample was collected, self-reported ancestry as: 15.7% German, 11.8% Irish, 9% English, 5.8% U.S., 5.1% Italian, 3.5% Polish, and 15.9% from other European areas (U.S. Census Bureau, Census 2000). Therefore, the Caucasian Americans in our study are likely to have multiple different ancestral countries of origin, which may reflect strata within the Caucasian groups.

*The magnitude of the potential effect of population structure.* Researchers suggest that the magnitude of bias caused by population stratification is not sufficient to significantly

bias results in association studies (Morton & Collins, 1998; Wacholder, Rothman, & Caporaso, 2000). However, a bias of 2-3% could lead to false-positive results (Rosenberg et al., 2002). Recent estimates of the increase in Type I error due to stratification culled from simulation studies range from 2% - 40% (Cardon & Bell, 2001; Cavali-Sforza, Menozzi, & Piazza, 1994; Gorroochurn et al., 2004).

The maximal inflation in Type I error in the simulation by Cardon and Bell (2001) occurs when the cases and controls show small differences in allele frequency. However, small differences in allele frequency between comparison groups reflect the magnitude of effect we hypothesize between the melanopsin gene and SAD. Cardon and Bell (2001) found that the ability to detect a 2% to 10% difference in allele frequency using 40 unlinked genetic markers is between 10% and 85%, with increased power to detect stratification increasing with allele frequency differences between comparison groups.

*Genomic Control of Population Stratification*

Genomic control of population stratification is a laboratory-based method for identifying latent strata that may exist in a sample, but are not clearly defined by demographic variables, and would be used for the present study in the event of positive associations. Genotype at many different polymorphic loci can be used to quantify the average amount of genetic variation due to differences between groups (Shriver et al., 2004). The quantified variation between groups in a sample can then be controlled for in analyses to remove the effects of 5-15% of the variation in allele frequency due to stratification (Shriver et al., 2004). Measuring the genetic variation due to group differences requires choosing polymorphic loci that are known to vary in frequency between groups, or Ancestry Informative Markers (AIMs; Shriver et al., 2005). Methods for controlling for population

structure rely on the assumption that population structure will be reflected by characteristic allelic patterns across the genome as a whole, whereas effects of candidate genes will be localized to disease associated gene regions (Pritchard & Donnelly, 2001).

*Number of markers required for genomic control.* European Caucasian populations vary between 1% and 3% genetically, and more heterogeneous populations vary between 10% and 30% (Pritchard & Donnelly, 2001). Risch et al. (2002) estimate that 20 carefully chosen SNPs are needed to distinguish African Americans from Asian Americans, and 40 SNPs are needed to distinguish Caucasian Americans from African Americans and Asian Americans. Distinguishing Caucasian Americans from Hispanic Americans is more difficult, requiring about 50 markers (Risch et al., 2002). Hundreds of markers are needed to differentiate strata within the same race, such as subgroups within Caucasian Americans. However, other researchers successfully distinguished African American, African Caribbean, and Hispanic American groups with 32 markers informative for ancestry (Hoggart et al., 2003). In general, 30-40 markers are needed to distinguish major racial groups (Freedman et al., 2004). Our proposal of using 30-40 markers may be sufficient to identify population stratification between racial groups, but not within the Caucasian group. Several hundred markers would be necessary to conclusively test for strata within the Caucasian group, which is outside of the scope of this proposal.

*Statistical approaches to genomic control.* Two methods have been proposed to statistically control for population stratification (Chen et al., 2003): (1) adjusting the test statistic by a factor estimated from the genomic markers (Devlin & Roeder, 1999) and; (2) identifying subgroups within study comparison groups and then performing tests of association while controlling for subgroup designation (i.e. structured association; Pritchard

& Donnelly, 2001). Other methods have been proposed, but have not yet been tested (Chen et al., 2003; Hoggart et al., 2003; Shriver et al., 2004). In comparisons using simulated data sets, the method proposed by Pritchard and Donnelly, structured association, is the most accurate with the highest power to identify population stratification (Hoggart et al., 2003). The computer program *structure* employs the structured association method and can be used to identify subgroups and stratify analyses on the basis of these subgroups (Pritchard & Donnelly, 2001). *Structure* uses a Bayesian approach to estimate the number of subpopulations and the probability of individual membership, or, in the presence of admixture, the probability that certain genes are from each subpopulation (Thomas & Witte, 2002). The proportion of ancestry from each group and likelihood of group membership can then be expressed as a covariate in regression models. In the present study, subgroups defined by demographic variables (i.e., Caucasian, female) can be analyzed separately in an attempt to test whether or not strata exist.

### *Alternate Genetic Study Methods*

As mentioned above, multiple methods exist for testing gene-disorder associations, and the alternate approaches are discussed here briefly to demonstrate the rationale for the particular approach chosen for the present study. Alternate approaches to identify gene-disorder associations such as genomic association and linkage analysis will not be used in this study because candidate gene analysis is methodologically better suited to study diseases in which multiple genetic factors are likely to account for a small proportion of risk individually (Risch & Merikangas, 1996). Linkage analysis involves analyzing polymorphic loci through the entire genome, and, therefore, would be most appropriate if the genetic factors of interest account for a large proportion of risk for a particular disorder (Risch &

Merikangas, 1996). In contrast, behavioral disorders are likely to involve multiple genetic risk factors that confer modest effects, and association testing is preferred to maximize power to identify genetic risk factors (Risch & Merikangas, 1996). Traditionally, linkage analysis has been used to identify LD between a hypothesized disease allele with another allele at a nearby location in the genome at a rate greater than chance, thereby supporting a role for the mutant allele in the disease (Gelehrter et al., 1998). Although LD will be exploited to identify haplotypes in the melanopsin gene, linkage analysis as a method is not proposed for this project. Candidate gene association studies have greater power than linkage analysis because the number of polymorphic sites in the genome is limited to candidate genes, reducing the number of tests and minimizing the likelihood of a false positive result (Risch & Merikangas, 1996). Linkage analysis requires much larger sample sizes than does association testing (Risch & Merikangas, 1996).

## Preliminary Data

Previously, the melanopsin gene was sequenced in a small number of individuals with SAD ($N = 25$) who were participants in a larger treatment study (Rohan, Tierney Lindsey, Roecklein, & Lacy, 2004). A sequence variation in melanopsin, rs2675703, was identified in this sample and sequenced in each participant. The rs2675703 variation, in which the more common C nucleotide is replaced with T, results in a substitution at the tenth amino acid in the amino terminus of melanopsin of a proline to a leucine and was therefore named "P10L." This P10L variant is potentially important for the function of the mature protein as the amino acid change is likely to impact the three dimensional structure of the expressed melanopsin protein. The location of the variant indicates it may disrupt trafficking of melanopsin to the cell membrane leading to premature degredation. Individuals with the P10L allele were over

3 times more likely to be in the SAD group than in the control group (*OR* = 3.07, 95% *CI* = 1.12–8.42). Comparison of the frequency of P10L in SAD and a convenience control sample of individuals (*N*=84) with an unrelated medical disorder, malignant hypothermia, demonstrated higher rates of the variant in the SAD group than in controls, $X^2$ (*df* 2, *N*=110)= 6.029, *p*= 0.049 (See *Table 1*).

*Table 1.* Frequency of genotype and alleles in the P10L variation in melanopsin

| | Genotype frequency; *n*(%) | | | | Allele frequency; *n*(%) | |
|---|---|---|---|---|---|---|
| | C/C | C/T | T/T | Any T (C/T & T/T) | C | T |
| Control *N* = 84 | 71(.85) | 12(.14) | 1(.01) | 13(.15) | 154(.92) | 14(.08) |
| SAD *N* = 25 | 16(.64) | 9(.36) | 0(.00) | 9(.36) | 41(.82) | 9(.18) |

Although these pilot data indicated that the P10L variant is present in the SAD sample, it was unclear if the variant segregates with SAD diagnosis given the small sample size and the possibility that the association could be due to demographic factors such as gender or ethnicity. Also, the control group was a group of individuals with malignant hypothermia (MH), rather than healthy controls. Individuals in the control/MH group were recruited for studies of an unrelated disorder and were not screened for depression and are expected to have a prevalence of SAD similar to the normal population. Therefore, the present study will use healthy controls, and have a sufficiently large sample size to test an association between melanopsin gene variations and SAD and seasonality.

## Study Purpose

A population-based case-control analysis was conducted to test for an association between the gene for melanopsin and SAD, using data gathered from 10 loci in and

surrounding the melanopsin gene. A combined haplotype-functional locus (fSNP) approach was used to determine the relative importance of fSNPs compared to specific haplotypes in associations with both SAD and seasonality. Sequence variants were genotyped including three coding variants (I394T, P10L, and D444G) found in coding regions of the melanopsin gene. Each of the coding variants in melanopsin was tested for an association with SAD or control status prior to performing the haplotype analysis. This step-wise approach allows distinction between possible effects of the individual coding variants compared to the effects of specific haplotypes in tests of association with SAD and seasonality.

*Hypotheses*

*Hypothesis 1.* SAD participants will demonstrate increased frequency of the fSNPs P10L, I394 and D444G variants relative to control participants. A larger proportion of SAD participants than controls are expected to have either the heterozygous or homozygous variant genotype as opposed to the more common homozygous wild-type genotype. Further, it is predicted that SAD cases will be more likely to have the less common allele than the more common allele at the fSNP loci when compared to controls.

*Rationale for Hypothesis 1.* This hypothesis is based on the central hypothesis that variations in the melanopsin gene may increase the risk of developing SAD such that individuals with SAD would be more likely to have functional variations in the gene for melanopsin compared to controls.

*Hypothesis 2.* Seasonality as measured by the Global Seasonality Score (GSS) of the Seasonal Pattern Assessment Questionnaire (Rosenthal et al., 1984a), will be associated with fSNP genotype (P10L, I394T, and D444G) in individuals with SAD. The effect of the age, gender, and self-reported ethnicity on the association between fSNPs and seasonality will be

tested. Analyses will be conducted within cases only, as individuals in the control group were selected from a different population and were selected for low levels of GSS (i.e., GSS $\leq 3$). The SAD cases have a range of GSS scores from 7 to 34.

*Rationale for Hypothesis 2.* Deficits in melanopsin may raise the threshold for adequate environmental light input such that low light availability in winter falls below the threshold required for euthymic functioning. This hypothesis proposes that melanopsin is related to both SAD and seasonality. Although individuals with SAD are likely to have higher global seasonality scores than individuals without SAD, the constructs of SAD and seasonality are defined differently. Whereas SAD is categorical, seasonality is continuous. Because common behavioral disorders such as SAD likely involve multiple genetic factors, individuals with SAD presumably vary in the number of risk factors they have, likely leading to varying degrees of trait expression depending on the cumulative effects of present risk factors (Plomin, DeFries, Craig, & McGuffin, 2003). Melanopsin variations associated with SAD may be responsible for variation in general seasonality in the population, including individuals who meet diagnostic criteria for SAD. Therefore, a measure of seasonality (GSS) will allow us to test the hypothesis that any identified melanopsin variations are associated with seasonality in a sample of individuals with SAD.

*Hypothesis 3.* Haplotypes constructed from information about variation in the melanopsin gene collected by genotyping will be associated with SAD diagnosis in a comparison of individuals with SAD and controls.

*Rationale for Hypothesis 3.* Specific haplotypes may be more or less common in individuals with SAD. As reviewed above, analyzing the gene for melanopsin with this haplotype-centric approach offers advantages to the SNP-centric approach by analyzing a

complete picture of the variation in the gene, which is more informative, robust and powerful (Niu, Qin, Xu, & Liu, 2002). Specific haplotypes associated with SAD diagnosis may reflect sequences in the melanopsin gene that may be etiologically important in SAD.

*Hypothesis 4.* Haplotypes constructed from information about variation in the melanopsin gene will be associated with degree of seasonality (GSS) in individuals with SAD.

*Rationale for Hypothesis 4.* The rationale for testing haplotypes in addition to fSNPs in the gene for melanopsin and the rationale for testing seasonality in addition to SAD diagnosis have been presented above. Hypothesis 4 extends both lines of investigation to testing for an association between degree of seasonality and specific haplotypes in individuals with SAD.

## Method

### *Participants*

DNA samples used in the present study are held by Dr. Robert Lipsky at the Laboratory of Neurogenetics at the National Institute of Alcoholism and Alcohol Abuse (NIAAA) and were gathered by researchers including Dr. Norman E. Rosenthal at the National Institutes of Mental Health (NIMH). Blood samples from individuals with SAD and healthy volunteers were originally collected between July 1992 and August 1999 to evaluate genes pertaining to the serotonin system in SAD (Enoch et al., 1999; Rosenthal et al., 1998; Sher et al., 1999). Control samples, recruited specifically for these previous NIMH studies, are a specific control sample matched to the SAD group on age, gender, and ethnicity.

*Inclusion and exclusion criteria.* SAD participants were recruited through the SAD outpatient clinic at NIH and were diagnosed according to DSM-III-R criteria (APA, 1987). Individuals learned about the SAD outpatient clinic through print advertisements in *The Washington Post* and self-referred for treatment and/or research to the clinic. Otherwise healthy, community adult volunteers underwent a diagnostic interview to rule out a history of Axis I disorders, the Structured Clinical Interview for DSM-III Axis I Disorders (Spitzer, Williams, Gibbon, & First, 1990). Inclusion criteria for the SAD group included SAD diagnosis, and they must be 18 years of age or older. Participants were excluded if they had another Axis I disorder such as an anxiety disorder.

Inclusion criteria for the control participants were no personal or family history of any Axis I disorder (i.e., clinical disorders such as mood and anxiety disorders, excluding personality disorders and mental retardation). The controls were gathered from the NIH Program for Healthy Volunteers, which volunteers can join by calling a phone number and completing a series of medical questionnaires. Currently, individuals can volunteer by completing an on-line questionnaire, however it is not documented how individuals were recruited at the time the participants in this study were enrolled (i.e., print or radio advertisements), or what specific medical questionnaires were required and were completed to establish control inclusion and exclusion criteria. Ideally, the method of recruitment and copies of the completed questionnaires or clinical interviews would be available to describe the control sample.

Participants were compensated $10 for the diagnostic interview, $10 for venipuncture, $20 for the first hour of their time and $10 for each hour thereafter, and were provided with $20 for an escort fee, if required for transportation to the NIH for the study. It

is important to note that the NIH volunteer pool used to enroll controls is a distinct recruitment method from that used to enroll SAD participants. Therefore, the recruitment methods are different between the case and control groups, limiting our ability to conclude that any observed findings are due to genetic rather than other confounding effects.

*Informed Consent and Identifying Information*

All participants were over the age of 18 when recruited. Participants had given informed consent for participation in the study including an explanation of the objectives, procedures, and the minimal risk associated. Participants were informed that their DNA would be identified by code, and that only the investigators would have access to the participants' names. For this proposal, no access to identifying information will be available, however, individual demographic data including age, ethnicity, and gender as well as questionnaire measure outcomes are linked to individual genetic samples. Participants were informed that their DNA and questionnaire data might be sent to other researchers, including for use in future studies to which they had not specifically given consent might be undertaken. Participants were informed they could withdraw from the study at any time, have their samples destroyed and identifying information removed.

## *Measures*

*Seasonal Pattern Assessment Questionnaire*

Participants completed the self-report Seasonal Pattern Assessment Questionnaire (SPAQ; Rosenthal et al., 1984a; Appendix), which contains a subscale measuring seasonality, the global seasonality scale (GSS). Participants rate 6 SPAQ items (sleep, appetite, mood, energy level, weight and social behavior) on a 5-point Likert scale for degree of change across the seasons ranging from 0 ("no change") to 4 ("extremely marked

change"), which are summed to derive the GSS. Therefore, the GSS can range from 0 to 24. Individual questions on the SPAQ address the above items comprising the GSS, as well as the following; months of year in which participants endorse feeling best/worst, gaining or losing the most weight, socializing most/least, sleeping most/least, eating most/least; how much weight fluctuates during the course of the year (from 0 to over 20 lbs.); how many hours per day the individual sleeps in winter, spring, summer, and fall; changes in food preferences across seasons; and, an appraisal of whether or not any changes in mood and behavior across the seasons represent a problem.

The SPAQ has good convergent validity with another self-report measure of seasonality, the Inventory for Seasonal Variation (ISV; Spoont, Depue, & Krauss, 1991) r = 0.63, which the authors suggest is a moderate correlation, and not higher, because the SPAQ and ISV differ in question format as opposed to content (Young, Blodgett, & Reardon, 2003). The internal consistency (Chronback's alpha) for the GSS subscale of the SPAQ is 0.81, with item correlations ranging from 0.48 to 0.75, in a general college student population (Young et al., 2003). Another study repeated a Chronbach's alpha of 0.85 for the GSS with item correlations ranging from 0.30 to 0.80 (Mersch et al., 2004b). The test-retest reliability for GSS over a 2-month interval is 0.76 in college students (Young et al., 2003). Over a follow-up interval of 5 to 8 years, the test-retest reliability of the GSS scale is 0.62 (Raheja, King, & Thompson, 1996). Over a follow-up period of 17 months, an Australian twin sample demonstrated a test-retest correlation of 0.58 on the SPAQ (Marriott, Armstrong, & Hay, 1994a). Therefore, the range of correlation coefficients for test retest reliability is between 0.51 and 0.76 for the GSS scale of the SPAQ. Factor analysis of the GSS scale items revealed a single factor accounting for 51.8% of the variance in SPAQ scores (Young et al.,

2003), replicating previous reports by Magnusson et al. (1997). When comparing a clinical interview for diagnosing SAD to the SPAQ criteria for estimating a SAD diagnosis (GSS ≥ 11, endorse a problem of moderate severity; Kasper et al., 1989), the SPAQ's sensitivity was 44% and specificity was 94% (Mersch et al., 2004a). These data suggest that the SPAQ is not a highly valid indicator of whether or not an individual actually has SAD; however, the current study uses the GSS to estimate severity of seasonality and does not use the SPAQ to diagnose SAD.

*Structured Clinical Interview for DSM-III-R Axis I Disorders (SCID-I)*

The Structured Clinical Interview for DSM-III-R Axis I Disorders (Spitzer et al., 1990) is a semi-stuctured interview for using the DSM-III-R criteria for the major Axis I diagnoses. The SCID-I was used here to assess for a lifetime diagnosis of Major Depression, Recurrent, with Seasonal Pattern and to rule out other Axis I disorders.

Test-retest reliability of the SCID-I was 0.64 and 0.73 for Major Depressive Disorder (MDD) over a period of 1-3 weeks and 7-10 days, respectively (Williams et al., 1992a; Zanarini & Frankenburg, 2001). Inter-rater reliability of two raters observing the same interview was high and ranged from 0.90 to 0.93 in three studies for the diagnosis of MDD (Segal, Kabacoff, Hersen, Van Hasselt, & Ryan, 1995; Skre, Onstad, Torgersen, & Kringlen, 1991; Zanarini & Frankenburg, 2001). To our knowledge, the reliability of the SCID-I in diagnosing the Seasonal Pattern Specifier, specifically, has not been tested, except as compared to other, less superior screening and diagnostic measures such as the SPAQ (Mersch et al., 2004b).

The convergent validity of the SCID-I has been tested by measuring the agreement between the SCID-I and a best estimate diagnosis such as 'LEAD' in which longitudinal

assessment (L), clinical interview by an expert (E), and all other data (AD) including family member interviews and medical records review are considered. Comparisons of LEAD and the SCID-I for DSM-III-R indicate good convergent validity (Fennig, Craig, Lavelle, Kovasznay, & Bromet, 1994; Kranzler, Kadden, Babor, Tennen, & Rounsaville, 1996; Kranzler et al., 1995; Ramirez Basco et al., 2000).

*Structured Interview Guide for the Hamilton Depression Rating Scale – SAD Version*

The Structured Interview Guide for the Hamilton Depression Rating Scale, SAD Version (SIGH-SAD; Williams, Link, Rosenthal, Amira, & Terman, 1992b; Appendix), is the most commonly used clinical assessment device for measuring severity of current SAD symptoms over the past week. The SIGH-SAD includes the 21-item Structured Interview Guide for the Hamilton Rating Scale for Depression (HAM-D; Williams, 1988) and a supplementary 8-item subscale to assess atypical depressive symptoms associated with SAD. The SIGH-SAD was administered to participants in this study during a current episode of SAD (for SAD participants) and at intake (for controls).

The HAM-D has been the gold standard for the assessment of depression for over 40 years (Bagby, Ryder, Schuller, & Marshall, 2004). A recent review of 70 studies on the psychometric properties of the HAM-D found that internal consistency ranged from 0.46 to 0.97, with 10 studies demonstrating internal reliability ≥ 0.70; Pearson's *r*, a measure of inter-rater reliability, ranged from 0.82 to 0.98 across studies; and test-retest reliability ranged from 0.81 to 0.98 (Bagby et al., 2004).

With regard to validity, the content of the HAM-D differs from DSM-IV criteria for MDD in that the HAM-D assesses some symptoms not included in DSM-IV criteria for depression (i.e., psychic anxiety, loss of insight, and hypochondriasis) and does not assess

feelings of worthlessness or difficulty concentrating which are in the DSM-IV criteria for a major depressive episode (Bagby et al., 2004). Convergent validity between the HAM-D and the Beck Depression Inventory – Second Edition (BDI-II; Beck, Steer, & Brown, 1996) ranged from 0.27 to 0.89, and convergent validity between the HAM-D and the major depression section of the SCID for DSM-IV (First, Spitzer, Gibbon, & Williams, 1995) was 0.37 in one study reviewed (Bagby et al., 2004). Although the SIGH-SAD is used in the present study as a measure of severity of depression symptoms as opposed to a diagnostic measure, 7 studies reviewed by Bagby et al. (2004) tested the ability of the HAM-D to discriminate between depressed and non-depressed individuals. Mean sensitivity was 0.76, mean specificity was 0.91, mean positive predictive value was 0.77, and mean negative predictive value was 0.92 (Bagby et al., 2004). Factor analysis in 15 studies reviewed by Bagby et al. (2004) identified between two and eight factors, including a sleep disturbance factor, a general depression factor, and an anxiety/agitation factor. The 8-item atypical symptom scale of the SIGH-SAD is highly and significantly correlated with similar items from the BDI-II (Meesters & Jansen, 1993). Other psychometric analyses have not been performed for the full SIGH-SAD.

### *Laboratory Methods*

Genomic DNA samples from participants with SAD and healthy volunteers has previously been collected and extracted from blood as part of the NIH protocol "Genetic Studies of Seasonal Affective Disorder and Non-Seasonal Affective Disorders." Lymphoblastoid cell lines were established with the collected samples for DNA extraction and samples have been detached from identifying information and are now stored in Dr. Lipsky's Laboratory of Neurogenetics.

Primers for each of the SNPs are based on the reference sequence for the melanopsin gene (GenBank accession number NM033282). The fSNPs are named for the amino acid the most common sequence codes for (i.e., P for Proline), followed by the position of the amino acid in the melanopsin protein starting from the 5' end (i.e., 10), followed by the amino acid the less common sequence codes for (i.e., L for Leucine) resulting in P10L, I394T and D444G for the three SNPs.

Polymerase Chain Reaction (PCR) amplification of the each region surrounding the fSNPs and the other 6-7 SNPs will be performed using the primer pairs. The PCR reaction for each SNP will consist of 100ng genomic DNA; 12.8 μM of each primer; 1.25 μM of each dATP, dCTP, dTTP, and dGTP; 10 mM PCR Buffer; 1.5 mM $MgCl_2$, and 1 U AmpliTaq Gold DNA Polymerase in a final volume of 25 μl. PCR, consisting of 34 cycles of annealing, will be performed at 65°C for 1 minute and extension performed at 72°C for 1 min 45 s. PCR will be terminated by extension at 72°C for 10 min.

Genotyping will be performed using a 5'-exonuclease assay with allele-specific fluorescence detection probes because this technique requires fewer steps than sequencing as was performed to genotype P10L. The 5'exonuclease assay uses fragments of DNA called probes that are about 13-20 bases long and will only bind to one specific sequence. The allele-specific probes are fluorescently labeled with distinct dyes such that a single signal will be observed when two alleles of one sequence are present, the other dye signal will be observed when two alleles of the other sequence are present, and a combined signal will be observed when one allele of each sequence is present. An assay to detect each allele will use fluorescently labeled probes with the dyes FAM and VIC at the 5'-end and linked to a nonfluorescent quencher (MGB) at the 3'-end. The quencher prevents fluorescence in the

absence of binding of the probe. The sequence of the allele specific probes will match the more or less common allele. The assays are based on the NCBI SNP Cluster ID's of each SNP and developed with Assays-on-Demand (Applied Biosystems, Foster City, CA). Genotyping reactions will be performed in 384-well plates using 10 ng genomic DNA, 0.5 μM of each amplification primer, 0.2 μM of probes, and 2.5 μl of Master Mix (Applied Biosystems, Inc.). The reaction thermal cycle will be 50°C for 2 min, 95°C for 10 min, 40 cycles of 95°C for 15 s, and Tm for each probe for 1 min. Tm for each probe will be determined once probes are designed to match the alleles of interest. Accuracy of genotyping will be verified by regenotyping 10% of samples, and accuracy and completion rates will be reported.

*Markers Selected to Construct Haplotypes*

SNPs chosen for markers in the study include those that are reported on the Single Nucleotide Polymorphism database (dbSNP), a public-domain archive for a broad collection of simple genetic polymorphisms. dbSNP distinguishes between information about SNPs submitted by individuals without evidence of validation, meaning that the polymorphism has only been observed in one DNA sample by the individual who submitted the data to the dbSNP database, and SNPs that have been validated by the submitter or by The National Center for Biotechnology Information (NCBI), the division of NIH that created the dbSNP database. NCBI also conducts research in computational biology, and may validate polymorphism through observing genotype or frequency data. Validation status is determined by the type of evidence used to confirm the variation and may be one of the following for a marker; (0) not validated, (1) validated by being observed by two independent submitters, one of which was experimentally validated, (2) submitter provided

the frequency or observed genotype of the polymorphism in a sample, (3) frequency data and independent experimental validation are both submitted, (4) the submitter used a second experimental validation method for the polymorphism, or (H) the polymorphism was genotyped by the HapMap project. The HapMap project goal is to create a haplotype map of the entire human genome using polymorphic data from four ethnically distinct populations; the Yoruba people in Nigeria (30 parent-child trios), Japanese in Tokyo (45 unrelated individuals), Han Chinese in Beijing (45 unrelated individuals), and Utah residents with ancestry from northern and western Europe (CEPH; 30 parent-child trios). Markers chosen for the present project are presented in Table 1, including the distance between markers and validation status for each marker.

Table 1

*Single Nucleotide Polymorphisms Used as Markers*

| RefSNP ID[a] | Location[b] | Chromosome Position[c] | bp to next SNP[d] | Validation Status[e] | Heterozygosity[f] |
|---|---|---|---|---|---|
| rs2675688 | flanking 5' | 88401431 | 3118 | 4, H | 0.34 |
| rs2675703 | Exon 1 | 88404549 | 1 | 2, H | 0.03 |
| rs11202106 | Exon 1 | 88404550 | 3154 | 0 | Unknown |
| rs10788521 | intron (2-3) | 88407704 | 1917 | 4 | 0.27 |
| rs3740335 | intron (5-6) | 88409621 | 2475 | 2 | 0.50 |
| rs1079610 | Exon 8 | 88412096 | 1376 | 4, H | 0.36 |
| rs12262894 | Exon 9 | 88413472 | 2550 | 0 | Unknown |
| rs3740341 | 3'UTR | 88416022 | 2293 | 0 | 0.36 |
| rs2803558 | 3'flanking | 88418315 | | 4 | Unknown |

[a]RefSNP ID number is a unique code assigned to each polymorphism.

[b]Location; flanking 5' region is before the gene, exons are coding regions of the gene, introns are non-coding regions of the gene between two exons, UTR is an un-translated region at the end of the gene, and 3' flanking region is after the gene.

[c]Chromosome Positions is the number of base pairs from the beginning of the chromosome to the marker in question.

[d]bp to next SNP is the number of base pairs between the current marker and the next marker selected for the haplotype analysis.

[e]Validation; please see text above for a description of the levels of validation with regard to the type of evidence submitted for validation of each marker.

[f]Heterozygosity is an estimate of how frequently an individual will have both the more and less common alleles, and higher degrees of heterozygosity is ideal in choosing markers because it indicates a likelihood that both alleles will be observed in the sample proposed for the present study, a condition necessary to determine genotype at a particular locus.

*Haplotype Reconstruction*

Haplotype blocks were constructed using genotype information from 7 markers described above in the melanopsin gene as well as the previously genotyped SNPs (rs2675703 and rs11202106) gathered from both SAD and control samples

Spacing 7 marker SNPs less than every 3kb (3,000 bases) apart in the gene for melanopsin constitutes a very dense map, denser than proposed maps with markers spaced every 5kb for fine mapping of the genome (Clark & Dean, 2004). Therefore, a densely mapped set of marker variants provided the opportunity to determine the extent of LD across the OPN4 gene, which was essential in establishing haplotype block structure. Based on the block structure, phase estimation and pairwise LD calculations was used to determine if one or more blocs were present (discussed below). The block structure was compared between SAD and control groups to determine if a different patterns of LD was observed in the case group (SAD). Differences in LD may predict population stratification. Alternatively, it may indicate the presence of risk or "protective" haplotypes associated with SAD or seasonality. Genomic control markers and structured association was not used to test for and control for

any population stratification because a significant association between haplotype structure and case status or seasonality was not found. Prior to reconstructing haplotypes from the marker genotypes, each marker was tested for deviation from HWE in the manner described below. However, the statistical method for haplotype reconstruction used in the present study has been tested and found to be robust to deviations from HWE (Stephens, Smith, & Donnelly, 2001). Reconstruction of haplotypes will be performed using (1) phase estimation and (2) pairwise LD computation, described below. Successive iterations of phase estimation and pairwise LD computation yield one haplotype block. The observed frequencies of each haplotype for each group, SAD and controls, was computed. After haplotype reconstruction, specific haplotypes from one or more iterations of haplotype reconstruction were tested for association with SAD diagnosis and seasonality as described in Hypotheses 3 & 4.

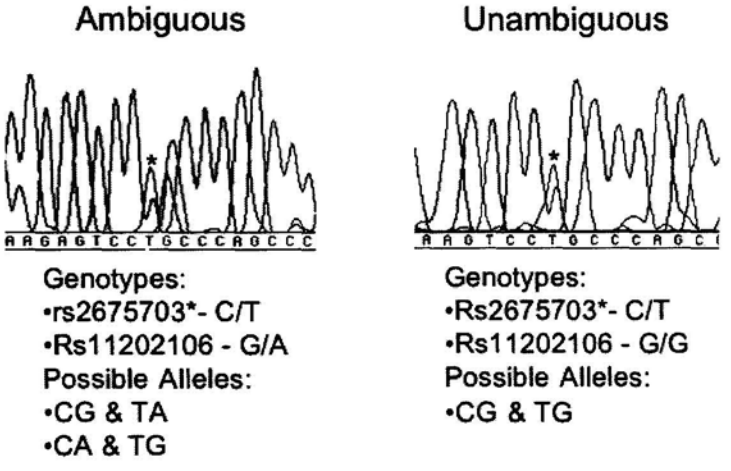
Two or more iterations of haplotype inference will be performed using the computer program PHASE 2.1 (Stephens et al., 2001). First, haplotype frequencies were estimated using all polymorphisms in each of the groups. These haplotypes were used for calculating the number of haplotypes for association analyses in Hypotheses 3 & 4. Second, a simplified haplotype estimation was performed omitting haplotypes with frequency less than 3% in the total sample.

*Phase Estimation*

In the case of the P10L SNP, rs2675703, and the immediately following SNP rs11202106, both loci were occasionally found to be heterozygous in a given sample. As each individual has two copies of the melanopsin gene (alleles), the 'phase' is ambiguous in these situations because four possible combinations exist that would result in the same

genotypes (see *Figure 2*). However, if an individual were homozygous at either locus, phase would be unambiguous, as shown in the right-hand panel of *Figure 2*.

*Figure 2.* Sequential variants in the melanopsin gene may or may not be in phase with each other on a given allele.




Determining phase directly through laboratory methods or by genotyping additional family members is costly and may be an inefficient use of resources because statistical methods for estimating phase exist (Stephens et al., 2001). Recent tests demonstrate that the Bayesian method proposed by Stephens et al. (2001) is the most accurate, reducing error rates by half when compared to other available algorithms for estimating phase including the Estimation-Maximization algorithm and Clark's algorithm (Stephens et al., 2001). Compared to two other Bayesian methods, the Stephens method is still superior in terms of error rate when tested using real and simulated data sets (Stephens & Donnelly, 2003). The Stephens method combines information about known haplotypes from unambiguous genotypes, with each haplotypes' likelihood of occurring, to assign the most likely haplotype for each individual based on a conditional distribution of unobserved haplotypes given observed data (Stephens & Donnelly, 2003). A freely available software program, PHASE

Version 2.1, was used to estimate the phase of ambiguous haplotypes that may arise given the genotype data to be collected (Stephens, 2003). PHASE Version 2.1 provided estimates of the frequency of each haplotype present in the data set for both populations separately, the SAD and control groups. These frequencies will be used in Hypotheses 3 & 4 below. PHASE v2.1 was run on all markers, as the pairwise LD analysis suggested that all markers were in high LD with one another.

*Pairwise LD Computation*

D', a commonly used measure of LD, is equal to 1 (absolute value) when there have been no recombination events or recurrent mutations between two polymorphic loci, indicating 'complete LD' (Carlson et al., 2004). In order to determine a haplotype block, a threshold of $|D'| \geq 0.9$ was set for this study to define areas showing low rates of structural changes within the study population.

As the haplotype structure of the melanopsin gene is currently unknown, markers spaced less than 3 kb apart in the gene were chosen and genotyped in order to determine the haplotype structure. Estimations of haplotypes created in PHASE can be directly input into the HaploBlockFinder V0.7 program which is designed to calculate rates of LD between pairs of markers using PHASE output (Zhang & Jin, 2003). Other commonly used programs for calculating pairwise LD use algorithms for estimating phase such as the EM algorithm and Clark's algorithm, but in the present study, using PHASE 2.1 and HaploBlock Finder V0.7 allows us to utilize the Bayesian algorithm for haplotype phase estimation. A measure of LD (D') was calculated from the expected haplotype frequency of two alleles ($P_{AB}$), and observed frequencies for each allele ($P_A$ & $P_B$) according to the equation $D = P_{AB} - (P_A \times P_B)$, with D' being the value of D normalized against the maximum value of D possible given the

individual allele frequencies (Xu et al., 2004). The program HaploBlockFinder V0.7 will be used to compute the absolute value of D' (|D'|) for each pair of SNPs (Zhang & Jin, 2003). Blocks were defined as a region in which all pairwise |D'| values are not lower than a certain threshold (initially set at 0.9).

*Successive Iterations of PHASE 2.1 and HaploBlockFinder V0.7*

Once the single haplotype block was defined, SNPs that uniquely distinguish common haplotypes (haplotype tagged SNPs, or htSNPs) were chosen by the HaploBlockFinder program such that a minimal set of htSNPs that uniquely distinguishes a certain percentage of all haplotypes was identified (Zhang & Jin, 2003). Frequency estimates provided by PHASE 2.1 are included in the tests of association in Hypotheses 3 & 4 in order to determine if the haplotypes defined by smaller subsets of htSNPs have increased power to describe an association between the gene for melanopsin and SAD or seasonality. Previous research has found that changing the number of SNPs included in haplotype association tests can identify a subset of SNPs that are associated with a given phenotype (Xu et al., 2004).

*Controlling for Population Stratification*

SAD participants are not significantly different from the control group on the basis of gender, $X^2$ (1, $N = 253$) = 3.33, $p \leq .10$, *ns* or race, $X^2$ (3, $N = 253$) = 2.41, $p \leq 1$, *ns*. However, ethnic and racial variations in genetic factors, as well as differences in environmental and cultural risk factors for SAD are likely to vary between different groups and may be evident when the proposed analyses described in the data analytic section for the present proposal are performed. Specifically, the initial analysis was performed using logistic regression, testing for potential interactions between self-reported race, age, gender and genotype/haplotype and the effect of genotype or haplotype. However, as reviewed

above, the use of genomic control markers can identify subgroups of individuals with genetic variation that differs from groups described by self-reported ancestry. Therefore, if significant associations between melanopsin and SAD or seasonality had been found, independent tests of population stratification would have been conducted. This would have comprised a set of 30-40 genetic markers that are unrelated to the gene of interest. However, as the results below are nonsignificant, genomic control for stratification is not indicated for the present study.

### *Data Analytic Strategy*

Haplotype analysis was be performed prior to testing Hypotheses 3 & 4. Deviation from Hardy-Weinberg equilibrium was tested prior to all tests of association. The data analytic strategy includes methods for testing for population stratification in the event of significant associations, and controlling for the effect of stratification in the analyses if population stratification is identified. However, this was not ultimately necessary for the present study due to the lack of significant associations.

### *Hardy-Weinberg Equilibrium*

Prior to tests of association, the distribution of genotypes and haplotype frequencies will be tested for Hardy-Weinberg equilibrium (HWE). Hardy-Weinberg equilibrium describes a state in which each genotype (i.e., both homozygous and heterozygous genotypes) are found in the frequency predicted by chance given the frequency of each of two alleles. Computing a $X^2$ statistic presupposes that each allele of a genotype be statistically independent and that deviations from independence (i.e., one genotype is more likely than predicted by chance) can inflate the Type 1 error rate (Schaid & Rowland, 1998). HWE is determined by the equation $p2 + 2\,p\,q + q2 = 1$ where $p$ is the frequency of the

more common allele in the population and $q$ is the frequency of the less common allele in the population. Testing for a deviation from HWE can identify a lack of independent segregation, nonrandom mating, a recent mutation that has not reached equilibrium, genotyping errors, bias in the selection of controls, or the existence of population stratification (Attia, Thakkinstian, & D'Este, 2003). However, in situations in which the less common allele exerts its effect on a disease phenotype only when homozygous (i.e., a recessive effect), deviation from HWE is to be expected (Fallin et al., 2001).

In the present study, each of the 9 markers including the fSNPs were tested for deviation from HWE. In the P10L association, cases and controls were tested for deviation from HWE in genotype distribution with Pearson's goodness-of-fit Chi-square ($df = 1$), the log likelihood ratio Chi-square ($df = 1$) and the Fisher exact test, all computed using the FINETTI program (Wienker & Strom, 2003). Close agreement between the three tests is expected unless the frequency of the less prevalent allele is very low, as is the case with P10L. In such cases, the asymptotic sampling distributions used by the Pearson Chi-square and Likelihood ratio tests are not appropriate, and Fisher's exact test is used. In the case of P10L, the exact test revealed that the predicted versus observed allelic frequency deviated from HWE in cases, $F(df = 1) = 0.194$, $p = 0.047$, but not in controls. In the proposed study, the statistical method for reconstructing haplotypes has been demonstrated to be robust to deviations from HWE (Stephens et al., 2001).

*Hypothesis 1: Data Analytic Strategy*

Hypothesis 1 was tested using logistic regression analysis to compare the frequency of genotypes and alleles found in SAD cases to control frequencies. Genotype (i.e., C/C, vs. C/T plus T/T for I394T and P10L; G/G vs. G/A plus A/A for D444G) was compared. In

addition, allele (i.e., C vs. T or G vs. A) frequencies were compared. Logistic regression is equivalent to the Pearson's chi-square ($X^2$) test and was used unless expected frequencies are less than 5 in more than 20% of total categories in which case the Fisher exact test was used (Xu et al., 2004). The SAD group was expected to have a higher frequency of the variant genotypes or less common allele in these analyses.

Analyses were completed twice, once with just genotype and group entered into the analysis, and a second time to test for potential interactions with type of SAD (i.e., unipolar, Bipolar I or Bipolar II), ethnicity, age, and gender. This allowed for testing whether or not type of SAD or self-reported ethnicity, age, or gender affected any identified association. Odds Ratio's were calculated for Hypothesis 1 because it was expected that individuals with P10L, I394T, or D444G would be more likely to be in the SAD group than the control group. For each analysis, effect size is reported. In addition, Cohen's (1988) definition of small (.1), medium (.3), and large (.5) effect size cut-offs for the effect size was applied.

*Hypotheses 2: Data Analytic Strategy*

Analysis of Covariance (ANCOVA) was used to test hypothesis 2 because this allows testing of a potential association between the fSNPs and degree of seasonality, in addition to testing potential interactions with gender, age, and/or race. ANCOVA was carried out using genotype at P10L, I394T, and D444G, as well as with specific haplotypes, as the independent variables and GSS as the dependent variable. Using ANCOVA allowed the test of the effect of type of SAD (i.e., unipolar, Bipolar I, or Bipolar II), age, gender, and ethnicity, allowing for adjustment of the effect of genotype on seasonality by these factors. Because the distribution of GSS within genotype groups is likely to deviate from normality, this was tested empirically. In addition, a histogram of GSS scores will be provided to evaluate

normality of the distribution. Unequal sample sizes in the different genotype and haplotype groups were tested, as this is a violation of an assumption of analysis of variance.

*Hypothesis 3: Data Analytic Strategy*

Two types of tests of association were used to test Hypothesis 3. The association analysis utility in PHASE 2.1 was used to compare the frequency distributions of haplotypes between the SAD cases and controls (Stephens et al. 2001). PHASE 2.1 estimates haplotype frequencies with a Bayesian-based algorithm and then uses a permutation test to determine the significance of difference in inferred haplotypes between cases and controls. In addition, PHASE provides estimates of the population frequency of haplotypes estimated in the haplotype reconstruction step, and these haplotype frequencies were compared in cases and controls with either logistic regression or a Fisher exact test. The exact test was used if 20% of the haplotype categories had an absolute count of 5 or fewer. In logistic regression or the exact test, the factors type of SAD (i.e., unipolar, Bipolar I, or Bipolar II), ethnicity, age, and gender were tested to see if they interacted with any identified effect of haplotype on SAD diagnosis. The direct analysis of frequencies of specific haplotypes was compared to the permutation-based test of association to determine whether or not the methods provide different results.

*Hypothesis 4: Data Analytic Strategy*

Each case's best-estimate haplotype and level of GSS was tested with ANCOVA for an association between haplotypes and seasonality. Interactions with type of SAD (i.e., unipolar, Bipolar I, or Bipolar II), age, gender, and self-reported ethnicity in the analysis were tested in a manner analogous to that proposed for Hypothesis 2.

*Ancillary Analyses*

Individuals in the SAD and control group completed the SIGH-SAD and SPAQ. It is possible that individual questions or categories of questions on these measures may be associated with the melanopsin gene, although specific hypotheses about which symptom categories or questions are associated with SNPs and/or haplotypes in melanopsin are not proposed. To test for possible associations, individual item responses on these questionnaires will be tested for an association with the SNPs and haplotypes in the melanopsin gene in ancillary tests.

A small subset of SAD participants ($n = 45$) were treated with light therapy and were analyzed to test an association between the melanopsin gene and response to light therapy. The light therapy remission rates are reported, including preliminary tests using the Fisher's Exact Test to identify any potential differences between genotype or haplotype groups and remission status (i.e., remitted or not) after light therapy. Records indicate that 45 SAD participants were treated with 10,000 lux of morning light for 2 weeks, during one of three consecutive winters, 1995-96, 1996-97, or 1997-98. Baseline and post-treatment SIGH-SAD assessments were used to determine remission status after 2 weeks of light therapy.

*Bonferroni Correction for Multiple Tests*

Because multiple tests of association are conducted on the same sample, a Bonferroni correction of the alpha level could be applied. Three fSNPs were tested for associations with both SAD diagnosis and seasonality (6 tests), and haplotypes were tested in two manners with both SAD diagnosis and seasonality (4 tests) yielding a total of 10 tests of association. The corrected threshold of significance for the fSNPs would be set at alpha = $0.05/n(m\text{-}1)$ where $n$ is the number of genetic factors tested (3 fSNPs) and $m$ is the number of alleles at each locus (2), yielding a corrected alpha-level of 0.017 for the fSNP analyses. However,

this correction may be overly punitive because it fails to consider that the three variants in melanopsin may be correlated, given that areas of the genome in close proximity to one another are likely to be inherited together (i.e., linkage; Newton-Cheh & Hirschhorn, 2005). Also, the number of specific haplotypes that will be identified is unknown and not corrected for in the above threshold of significance. The degree to which one variant is correlated with another varies from one region of the genome to another (Twells et al., 2003), and it is unknown if any of the melanopsin variants being studied are correlated or if they are all independent. Correlation between the genotypes will be empirically tested through computing pair-wise LD (D') to determine if the variants are independent. If so, the corrected alpha-level will be used, including correction for the number of independent haplotypes identified. Given the relatively short length of the melanopsin gene (11.8 kb; Provencio et al., 2000), we expect few distinct blocks and high LD across the gene.

*Power Estimate*

Schork et al. (2000) found that, given a minor allele frequency of between 10% and 25%, alpha level of 0.05, and between 50% and 75% correlation (LD) between markers and putative disease loci, a sample of 24-229 would be required for 80% power. The range in required sample size is due to the possibility of differing magnitudes of effect and different possible models of inheritance (dominant, recessive, or additive). The proposed study makes use of very densely spaced markers likely to be more than 50% correlated with any disease locus in the melanopsin gene.

In the logistic regression analysis proposed for testing an association between fSNPs and SAD diagnosis, power can be estimated using the expected frequency of the risk conferring allele and the estimated odds ratio. In this case, the frequency of the risk

conferring allele of 22%, the frequency obtained in the control group for the P10L analysis. With the available sample size of 222, we have 80% power to detect an effect with an odds ratio of 1.65. An odds ratio of between 1.5 and 2.0 is likely for this study. We would have 63% power to detect an odds ratio of 1.5, and 96% power to detect an odds ratio of 2.0 with a sample of 222 individuals (Hsieh, 1989). A limitation of this power estimate is that the frequency of other risk conferring alleles may not be similar to the 22% found for P10L.

Tab

## Results

### *Participants*

Participants in the SAD group were 70% female, 95% Caucasian, 5% African American, and 1% Asian American, and were predominately middle aged ($M = 40.1$, $SD = 8.3$, minimum age = 18, maximum age = 66). Participants in the SAD group had an average global seasonality score (GSS) of 16.6 ($SD = 3.3$), with a minimum of 8 and a maximum of 24, and these data were normally distributed. 53% of the SAD group had unipolar Major Depressive Disorder, 2% had Biploar II Disorder, 17% had Bipolar I Disorder, and the remaining 28% were missing data on this variable.

Documentation regarding the previous studies completed with this sample state that the control group was matched to the case group, however it is unclear how participants were matched, as the sample sizes are not equivalent. Ideally, the matching strategy would be known and could be presented, and the groups would not differ on the basis of age, gender, ethnicity, or other potentially stratifying variables such as socio-economic status. Participants in the control group were 58% female, 97% Caucasian, 3% African American, and were predominately middle aged ($M = 39.8$, $SD = 10.7$, minimum age = 19, maximum

age = 61). Controls had a maximum GSS of 10, and a minimum of zero ($M = 2.23$, $SD = 2.01$). The SAD and control groups were not significantly different on the basis of age [$F(1, N = 228) = 0.063$, $p = 0.80$], gender [$X^2(1, N = 228) = 3.45$, $p = 0.066$], or ethnicity [$X^2(2, N = 228) = 1.108$, $p = 0.60$]. However, there was a marginally significant difference ($p = 0.07$) between groups in gender, with 70% of the SAD group being female, and only 58% of the controls being female. Because of this marginal gender difference, gender was included as a covariate in logistic regression analyses below.

Table 2. Demographic variables for the SAD and control groups.

| $N = 253$ | SAD Group ($n = 154$) | Nondepressed Control Group ($n = 99$) |
|---|---|---|
| Gender | | |
| Female | 106 (.69) | 57 (.58) |
| Male | 48 (.31) | 42 (.42) |
| Ethnicity | | |
| Caucasian | 141 (.92) | 95 (.96) |
| African American | 6 (.04) | 2 (.02) |
| Hispanic or Latino | 5 (.03) | 2 (.02) |
| Asian American | 2 (.01) | 0 (.00) |
| Other | 0 (.00) | 0 (.00) |
| Seasonality (GSS) *M* (*SD*) | 16.6 (3.3) | 2.23 (2.01) |

*Sequencing Success*

Failure rates and error rates were calculated for the two markers sequenced by direct sequencing, P10L and I394T. A total of 231 samples were sequenced for the P10L marker, and of those, 7 contained DNA at concentrations too low to yield sequence. Of the remaining 224, 17 samples failed to yield useable sequences (failure rate = 7.6%). 15% of these samples were repeated and no errors were identified. For the I394T marker (rs1079610), 224 samples were sequenced with 11 failing to yield useable sequences (failure

rate = 4.9%). 15% of the samples were sequenced twice, and 3 samples were in error (error rate = 9%), below the previously established limit.

### *Hardy-Weinberg Equilibrium*

Prior to tests of association, the distribution of genotypes at each locus was tested for Hardy-Weinberg Equilibrium (HWE). Genotypes at the loci P10L and I394T were tested, but not D444G because this locus was monomorphic in the present sample. We used the FINETTI program (Wienker & Strom, 2003) to compute Pearson's goodness-of-fit Chi-square, the log likelihood ratio Chi-square, and, in certain cases, the Fisher's exact test to compare observed frequencies from those expected given HWE. Fisher's exact test was used when asymptotic sampling distributions are present, as in the case of P10L. Neither controls nor the SAD cases differed from HWE at any of the 10 markers tested.

### *Hypothesis 1 Results*

In Hypothesis 1 it was proposed that SAD participants would demonstrate an increased frequency of the coding variants P10L, I394T, and/or D444G relative to control participants.

*P10L: Hypothesis 1*

In the case of P10L, the distribution of all three genotypes (C/C, C/T, and T/T) between the SAD and control groups was compared using Fisher's Exact Test, $X^2(2, N = 228) = 5.09, p = .09$, *ns*. Allelic Frequency tested with Fisher's Exact Test was also not statistically different between groups at the P10L locus, $X^2(1, N = 456) = 2.29, p = .13$.

Upon observing that all seven individuals with the T/T genotype at P10L were in the SAD group, and there was little difference between groups in the frequency of the C/T genotype, a post-hoc "autosomal recessive disorder" interpretation was considered. The term

"recessive" refers to a disorder or phenotype that is only seen with the homozygous genotype (an organism that has two copies of the same allele) and never in a heterozygous genotype on the autosomes, the non-sex chromosomes. Previously identified recessive retinal disorders include Stargardt's Disease or Fundus Flavimaculatus, Leber's Congenital Amaurosis, and a recessive form of glaucoma called Primary Congenital Glaucoma. If this is the case with P10L and SAD, the homozygous T/T genotype would be more common in SAD patients than in controls, but patients and controls would not necessarily differ in their frequency of the C/T genotype. To test the autosomal recessive disorder hypothesis, a 2x2 Fisher's Exact Test comparing the SAD and control groups on the frequency of T/T vs. the combined frequency of C/T and C/C was conducted. SAD participants had a higher frequency of the homozygous minor allele (T/T) genotype than controls, when compared to the combined frequency of C/C and C/T together, Fisher's Exact test (1, N = 220) = 4.38, p = .047. However, only 7 (5%) of SAD participants had the T/T genotype compared to zero controls. This comparison is illustrated in the shaded area of Table 3.

*Table 3.* Frequency of genotype and alleles at the P10L locus

| | Genotype frequency, *n* (%) | | | | Allele frequency, *n* (%) | |
|---|---|---|---|---|---|---|
| | C/C | C/T | T/T | C/T and C/C | C | T |
| SAD (*N* = 132) | 96 (73%) | 29 (22%) | 7 (5%) | 125 (95%) | 221 (86%) | 36 (14%) |
| Control (*N* = 90) | 70 (78%) | 20 (22%) | 0 (0%) | 90 (100%) | 160 (89%) | 20 (11%) |

The effect size for the one-degree of freedom Fisher's Exact test of P10L genotype (T/T vs. C/C+C/T) is $d = 0.46$, computed using the Arcsine test (Lipsey & Wilson, 2001). According to Cohen's (1988) definition of small (0.2), medium (0.5), and large (0.8) effect

sizes based on values of *d*, the effect size for this comparison is medium. The Odds Ratio is another measure of effect size used for Fisher's Exact Test for this comparison, *OR* = 5.6336 [95% CI 1.2201 - 26.0113], indicating that individuals with the T/T genotype are 5.6 times more likely to be in the SAD group than the control group. The Peto method of calculating an odds ratio, used for the present analysis, is better than the other approaches at estimating odds ratios when there are trials with no events in one or both arms (Chalmers, Enkin, & Keirse, 1989). This Peto Odds Ratio test is available on-line (http://www.hutchon.net/ConfidORnulhypo.htm).

Logistic regression was performed with gender, race, and age included as covariates in the analysis. There was no risk associated with P10L genotype (i.e., C/C, C/T, or T/T dummy coded) in logistic regression, *OR* = 1.553 (95%CI 0.901 – 2.677), $p = .113$. Effect size for the logistic regression analysis is expressed as the Odds Ratio, and is not significantly different from 1 in the present analysis. Logistic regression testing the effect of having zero, one, or two of the minor alleles (i.e., the T allele at P10L), including demographic covariates, was not statistically significant, *OR* = 1.669 (95% *CI* 0.94 – 2.97), $p = 0.081$. The Odds Ratio is not significantly different from 1 in this analysis either.

*I394T: Hypothesis 1*

The frequency of genotypes and alleles at the I394T locus is reported below in Table 3. Fisher's Exact Tests were used for three comparisons. Tests of the genotype frequency [$X^2(2, N = 217) = 1.50, p = .50$], least and most common allele genotypes [i.e., T/T vs. C/T+C/C; $X^2(1, N = 217) = 1.32, p = .26$], and allelic frequencies [$X^2(1, N = 434) = 0.668, p = .42$] were not significant. In addition, I394T genotype did not predict group membership in logistic regression (*OR* = 0.933 [95%CI 0.635 – 1.371], $p = .725$, when including age,

gender, and race as covariates in the analysis. Logistic regression testing the effect of having zero, one, or two copies of the minor or less common allele (i.e., the C allele at I394T), including demographic covariates, was nonsignificant, $OR = 0.805$ (95% $CI$ 0.531 – 1.218), $p = 0.304$. The Odds Ratio or effect size is not significantly different from 1 in this analysis or in the above logistic regression.

*Table 3*. Genotype and allelic frequencies at the I394T locus

| | Genotype frequency *n* (%) | | | | Allelic frequency *n* (%) | |
|---|---|---|---|---|---|---|
| | C/C | C/T | T/T | C/C and C/T | C | T |
| SAD (*N* = 128) | 16 (13%) | 56 (44%) | 56 (44%) | 72 (56%) | 88 (34%) | 168 (66%) |
| Control (*N* = 89) | 11 (12%) | 46 (52%) | 32 (36%) | 57 (64%) | 68 (38%) | 110 (62%) |

*D444G: Hypothesis 1*

The D444G locus in the melanopsin gene was found to be monomorphic in the present sample, with all participants having the homozygous genotype for the major allele (C). This is consistent with data reported on the frequency of polymorphisms at this site (very low) in Caucasian samples obtained by multiple laboratories and accessed through on-line databases. Therefore, the analysis comparing SAD participants to controls on D444G was not performed.

*Hypothesis 2 Results*

ANCOVA was used to test an association between fSNPs (P10L and I394T) and seasonality, while controlling for potential interactions with gender, age, type of SAD (i.e., unipolar, Bipolar I, or Bipolar II), and/or race and adjusting for the effect of these factors on the association between genotype and seasonality among the SAD cases. As described

above, the D444G locus in the melanopsin gene was found to be monomorphic in the present sample, with all participants having the homozygous genotype for the major allele (C). Therefore, the analysis testing an association between D444G and seasonality was not performed.

*P10L: Hypothesis 2*

The ANCOVA testing the association between P10L genotype and seasonality, with age, gender, race, and diagnosis among SAD patients was nonsignificant, $F(2,94) = 1.330$, $p = 0.270$. However, age was a significant predictor in the relationship ($p = 0.036$). The parameter estimate for age was negative, indicating an inverse association between age and the dependent variable, seasonality. Levene's test of equality of error variances was nonsignificant, supporting the hypothesis of equality of variance of the dependent variable, seasonality, across our three P10L genotype groups. Tests of Normality (e.g., Shapiro-Wilk) supported the hypothesis that the data in each cell of the association reflects a normal distribution.

Among individuals with SAD, differences in global seasonality scores between the mood disorder diagnostic groups were not statistically different. Individuals with Bipolar II type SAD had the highest average GSS ($n = 21$, $M = 18.29$, $SD = 0.578$), followed by individuals with unipolar SAD ($n = 71$, $M = 16.51$, $SD = 0.373$), and individuals with Bipolar I type SAD had the lowest levels of seasonality ($n = 3$, $M = 13.33$, $SD = 1.764$).

*I394T: Hypothesis 2*

The ANCOVA testing the association between I394T genotype and seasonality among SAD patients, with age, gender, race, and diagnosis as covariates was nonsignificant, $F(2, 86) = 1.745$, $p = .181$. Levene's test of equality of error variances was nonsignificant,

supporting the hypothesis of equality of variance of the dependent variable, seasonality, across the three I394T genotype groups. Tests of Normality (e.g., Shapiro-Wilk) supported the hypothesis that the data in each cell of the association reflects a normal distribution.

### *Hypothesis 3 Results*

An association between haplotypes and case status (i.e., control or SAD) was tested in two ways, first with an automatic association analysis utility in PHASE 2.1, and second with Fisher's Exact Test and logistic regression. The test in PHASE 2.1 is termed the 'permutation test of association' because PHASE 2.1 includes estimates of confidence in the phase estimation in multiple (permuted) tests of association. The 'direct test of association' is carried out using the SPSS and StatXact statistical applications and is not permuted. Before presenting the results of the haplotype tests of association, the results of the phase estimation and haplotype identification procedures performed with the HaploView utility are presented.

#### *One Haplotype Block Identified: Hypothesis 3*

The HaploView utility calculated parwise rates of linkage disequilibrium (LD) between each pair of the 10 markers used in the present analysis. D' and LOD scores for each parwise test identified LOD was greater than 2 and D' was exactly 1 for each pair of markers, suggesting only one haplotype block is present across the entire gene. This means that there is no evidence within the gene for a recombination site, or a location that has experienced chromosomal crossing over in recent history. This is important for our haplotype tests of association because it means that each haplotype, even though it reflects the pattern of variation across all 10 of our markers, can reliably be considered a single event of inheritance, rather than two or 10 events. If we are to apply Bonferroni corrections, we

will consider each test of the identified haplotypes as one test, rather than as multiple tests of between 2 and 10 tests of association. Therefore, there is evidence that each haplotype is one event of inheritance, the correction for multiple tests is minimized.

*Six Haplotypes Identified with PHASE 2.1: Hypothesis 3*

Initially, the PHASE 2.1 program identified 16 haplotypes, although 10 of these were rare (i.e., < 2% frequency). Individuals with rare haplotypes were removed from further analyses, yielding 190 individuals for the final haplotype analyses. Haplotypes 1 and 2 contain the coding variant at the I394T locus, and haplotype 5 contains the coding variant at the P10L locus. Of the rare haplotypes deleted from the final analyses, the majority (70%) had neither the P10L or I394T coding variant. Of the deleted samples, 8% had the P10L variant, 20% had the I394T variant, and 2% had both. Therefore, it is possible, but unlikely, that the rare haplotypes removed impacted the test of association by removing potentially important variants.

*Permutation Test of Association: Hypothesis 3*

The PHASE 2.1 utility automatically runs a case-control significance test of association with the 6 identified haplotypes, taking into account the certainty of the phase estimates calculated by PHASE 2.1. The $p$ value associated with this test was 0.39, *ns*.

*Direct Tests of Association: Hypothesis 3*

An initial Fisher's Exact Test comparing the distribution of all 6 haplotypes across SAD and control groups identified no significant difference between groups, $X^2(5, 378) = 3.75, p = 0.58$, *ns*.

Table 4. Distribution of Identified Haplotypes Across SAD and Control Groups

| | hap1 | hap2 | hap3 | hap4 | hap5 | hap6 | totals |
|---|---|---|---|---|---|---|---|
| SAD | 65 (30%) | 12 (6%) | 7 (3%) | 71 (33%) | 29 (13%) | 33 (15%) | 217 |
| Control | 56 (35%) | 9 (6%) | 7 (4%) | 55 (34%) | 19 (12%) | 15 (9%) | 161 |
| Totals | 121 (32%) | 21 (6%) | 14 (4%) | 126 (33%) | 48 (13%) | 48 (13%) | 378 |

To test an association with logistic regression among SAD cases, controlling for age, gender, race, and diagnosis, the haplotypes were dummy coded by hand to reflect the frequency of each of the 6 haplotypes for each participant. None of the covariates predicted SAD or control group membership, and the frequency of the 6 haplotypes did not predict group membership either (i.e., SAD or control). Odds Ratio's, 95% confidence intervals, and *p* values are reported below for the haplotype frequency variables.

Table 5. Results of Logistic Regression for Association Between Haplotypes and Group

| Haplotype # | Odds Ratio | Lower Bound | Upper Bound | *p* value* |
|---|---|---|---|---|
| 1 | 0.609 | 0.035 | 10.492 | 0.733 |
| 2 | 0.538 | 0.028 | 10.355 | 0.681 |
| 3 | 0.738 | 0.036 | 15.178 | 0.844 |
| 4 | 0.534 | 0.030 | 9.446 | 0.669 |
| 5 | 0.385 | 0.021 | 6.967 | 0.518 |
| 6 | 0.295 | 0.015 | 5.726 | 0.420 |

*All *p*'s are nonsignificant (*ns*).

*Hypothesis 4 Results*

In Hypothesis 4, it was proposed that one or more of the 6 specific haplotypes identified would be associated with seasonality (GSS) in individuals with SAD, while controlling for the effect of age, gender, race, or mood disorder diagnosis (i.e., unipolar, Bipolar I or Biploar II type SAD). The haplotypes were dummy coded by hand to reflect the frequency of each of the 6 haplotypes for each participant, and race and diagnosis were dichotomized for ease of analysis (i.e., white or non-white, and unipolar or any type of Bipolar disorder, respectively). The ANCOVAs testing the association between haplotype

frequencies and seasonality among SAD patients were all nonsignificant, and F values, *p* values, and the effect size, Partial Eta squared effect size are reported below. Levene's test of equality of error variances was significant ($p = 0.003$), threatening the assumption of equality of variance of the dependent variable, seasonality, across the haplotype groups. This is a limitation of this analysis.

Table 6. Association between Haplotype Frequencies and Seasonality (GSS)

| Haplotype # | *df* | *F* | *p* value* | *Partial Eta*$^2$ |
|---|---|---|---|---|
| 1 | 2 | 0.642 | 0.530 | 0.021 |
| 2 | 1 | 0.448 | 0.506 | 0.007 |
| 3 | 1 | 0.185 | 0.669 | 0.003 |
| 4 | 2 | 0.160 | 0.853 | 0.005 |
| 5 | 2 | 0.531 | 0.591 | 0.017 |
| 6 | 2 | 1.973 | 0.148 | 0.062 |

*All *p*'s are nonsignificant (*ns*).

*Ancillary Analyses*

Questionnaire measures including the SIGH-SAD and SPAQ were used to test whether or not individual questions or categories of questions are associated with any melanopsin gene variants P10L and I394T and each of the 6 haplotypes. To correct for the bias of multiple tests, only those with a *p* value of less than 0.005 are reported here. The number of copies of a particular haplotype are reported for haplotype analyses in the comparison column (e.g., 0, 1, or 2 copies of haplotype 1). Some individual SPAQ and SIGH-SAD items were associated with specific haplotypes. Neither P10L or I394T was associated with any items on these measures. The variants and haplotypes did not related to the typical depressive symptoms (21-item Hamilton Depression Rating Scale; HAM-D) or the atypical depressive symptoms (8-item atypical subscale) when the SIGH-SAD was divided into it's component parts.

Table 7. Items on the SIGH-SAD and SPAQ associated with melanopsin variations.

| Variation | Questionnaire | *F* | *p* value | comparison[9] | *M* | *SD* |
|---|---|---|---|---|---|---|
| Hap 1 | SPAQ Dry Days[1] | 5.965 | 0.004 | 0, *n*=42<br>1, *n*=23<br>2, *n*=7 | 6.119<br>5.696<br>4.500 | 1.131<br>1.145<br>0.548 |
| Hap 3 | SIGH-SAD Hypochondriasis[2] | 12.600 | 0.001 | 0, *n*=27<br>1, *n*=3 | 0.333<br>1.667 | 0.555<br>1.155 |
| Hap 3 | SIGH-SAD Diurnal A[3] | 10.000 | 0.004 | 0, *n*=27<br>1, *n*=3 | 1.444<br>0.333 | 0.577<br>0.577 |
| Hap 3 | SIGH-SAD Depersonalization[4] | 15.448 | 0.001 | 0, *n*=27<br>1, *n*=3 | 0.185<br>1.670 | 0.483<br>1.528 |
| Hap 4 | SIGH-SAD Agitation[5] | 6.711 | 0.004 | 0, *n*=15<br>1, *n*=14<br>2, *n*=1 | 0.133<br>0.286<br>2.000 | 0.352<br>0.611<br>0.000 |
| Hap 6 | SIGH-SAD Increased Appetite[6] | 8.522 | 0.001 | 0, *n*=20<br>1, *n*=9<br>2, *n*=1 | 0.450<br>2.000<br>0.000 | 0.759<br>1.323<br>0.000 |
| Hap 6 | SIGH-SAD Increased Eating[7] | 6.513 | 0.005 | 0, *n*=20<br>1, *n*=9<br>2, *n*=1 | 0.500<br>1.667<br>0.000 | 0.761<br>1.000<br>0.000 |
| Hap 6 | SPAQ Humid Weather[8] | 6.081 | 0.004 | 0, *n*=50<br>1, *n*=18<br>2, *n*=4 | 3.360<br>4.778<br>2.500 | 1.454<br>2.102<br>1.291 |

Notes:

SPAQ = Seasonal Pattern Assessment Questionnaire (Rosenthal et al., 1984a).

SIGH-SAD = Structured Clinical Interview Guide for the Hamilton Depression Rating Scale, Seasonal Affective Disorder Version (Williams et al., 1992b).

[1]SPAQ Dry Days - "Indicate how the following weather (dry days) makes you feel, from 3 = in very low spirits or markedly slowed down, to +3 = markedly improves your mood or energy level."

[2]SIGH-SAD Hypochondriasis - "In the last week, how much have your thoughts been focused on your physical health or how your body is working (compared to your normal thinking)? (Have you worried a lot about being or becoming physically ill? Have you really been preoccupied with this?) Do you complain much about how you feel physically? Have

you found yourself asking for help with things you could really do your self? IF YES: Like what, for example? How often has that happened? 0 = not present; 1 = self-absorption (bodily); 2 = preoccupation with health; 3 = frequent complaints, requests for help, etc.; 4 = hypochondriacal delusions.

[3]SIGH-SAD Diurnal Variation A - "Over the past week, in the first few hours after waking up, have you been feeling better, worse, or no different from before you go to sleep?" 0 = no variation OR not currently depressed; 1 = worse after awakening; 2 = worse before going to sleep.

[4]SIGH-SAD Depersonalization and Derealization - "In the past week, have you ever suddenly had the sensation that everything is unreal, or you're in a dream, or cut off from people in some strange way? Any spacey feelings? IF YES: Tell me about it. How bad has that been? How often this week has that happened?" 0 = absent; 1 = mild; 2 = moderate; 3 = severe; 4 = incapacitating.

[5]SIGH-SAD Agitation - RATING BASED ON OBSERVATION INTERVIEW. IF TELEPHONE INTERVIEW: "As we talk, are you fidgeting at all, or having trouble sitting still? For instance, are you doing anything like playing with your hands or your hair, or tapping your foot? Do others notice that you are restless?" 0 = none; 1 = fidgetiness; 2 = playing with hands, hair, etc.; 3 = moving about, can't sit still; 4 = hand-wringing, nail biting, hair-pulling, biting of lips.

[6]SIGH-SAD Increased Appetite – "In the past week, has your appetite been greater than when you feel well or OK? IF YES: Do you want to eat a little more, somewhat more, or much more than when you feel well or OK?" 0 = no increase in appetite; 1 = wants to eat a

little more than usual; 2 = wants to eat somewhat more than usual; 3 = wants to eat much more than usual.

[7]SIGH-SAD Increased Eating – "In the past week, have you actually been eating more than when you feel well or OK? IF YES: A little more, somewhat more, or much more than when you feel well or OK?" 0 = is not eating more than usual; 1 = is eating a little more than usual; 2 = is eating somewhat more than usual; 3 = is eating much more than normal.

[8]SPAQ Humid Weather "Indicate how the following weather (humid weather) makes you feel, from 3 = in very low spirits or markedly slowed down, to +3 = markedly improves your mood or energy level."

[9]The copies of a particular haplotype are reported for haplotype analyses in the comparison column (e.g., 0,1, or 2 copies of haplotype 1).

*Light Therapy Remission Rates*

Data were obtained for 45 individuals with SAD who were included in the above study and who completed the SIGH-SAD before and after a 2-week trial of light therapy. The following criteria were used to define SAD episode remission (Terman et al., 1990): (1) pre- to post-treatment reduction in total SIGH-SAD score by at least 50%, HAM-D score $\leq 7$, and atypical score $\leq 7$, or (2) HAM-D score $\leq 2$ and atypical score $\leq 10$. Twenty-four out of 45 individuals met one or both criteria for remission (53% remission rate), and a majority of those had successful sequences for P10L and I394T as well as the haplotype analyses. Although there are too few individuals for a conclusive test of associations between light therapy remission status and melanopsin gene variations, summary data are reported below in Table 8.

Table 8. Remission status of 45 participants who completed a 2-week trial of light therapy by melanopsin gene variants and haplotypes.

| Locus | P10L | | | I394T | | |
|---|---|---|---|---|---|---|
| Genotype | C/C | C/T | T/T | C/C | C/T | T/T |
| Remitted ($n$ = 24, 28)[1] | 17 (76%) | 6 (19%) | 1 (5%) | 0 (0%) | 12 (57%) | 9 (43%) |
| Not Remitted ($n$ = 21, 23)[1] | 16 (71%) | 4 (25%) | 1 (4%) | 3 (12%) | 6 (25%) | 13 (54%) |
| | Haplotype | | | | | |
| | 1 | 2 | 3 | 4 | 5 | 6 |
| Remitted ($n$ = 46) | 11 (24%) | 2 (4%) | 0 (0%) | 17 (37%) | 6 (13%) | 10 (22%) |
| Not Remitted ($n$ = 38) | 9 (24%) | 2 (5%) | 2 (5%) | 12 (32%) | 7 (18%) | 6 (16%) |

Note: Data are presented as number (percentage). SIGH-SAD Remission Criteria = (1) pre- to post-treatment reduction in total SIGH-SAD score by at least 50%, HAM-D score $\leq 7$, and atypical score $\leq 7$, or (2) HAM-D score $\leq 2$ and atypical score $\leq 10$ (Terman et al., 1990).

[1]The n's are reported for P10L and I394T analyses, respectively.

Fisher's exact tests yielded no significant differences between the observed frequencies of melanopsin gene variants (i.e., P10L, I394T, or haplotypes) between individuals who did vs. did not fully remit with light therapy. There were two individuals treated with light who had the T/T P10L genotype, with one experiencing a remittance of SAD symptoms, and one not experiencing remittance after light therapy.

## Discussion

### *Summary of Results*

This study tested whether coding variants or single nucleotide polymorphisms (SNPs) in the melanopsin gene differentially relate to seasonal affective disorder (SAD) diagnosis or

no personal or family history of Axis I psychopathology (controls). Three identified SNPs (i.e., P10L, I394T, and D444G) were examined. In the case of P10L, the overall distribution of the C/C, C/T, and T/T genotypes did not differ between SAD and control participants. However, SAD participants were found to have a higher frequency of the homozygous minor allele (T/T) genotype ($n$ = 7, 5%) than controls ($n$ = 0, 0%), but only when compared to the combined frequency of C/C and C/T genotypes. The effect size for this comparison is medium, and the Peto odds ratio indicated that individuals with the T/T genotype are 5.6 times more likely to be in the SAD group than the control group. This is what is referred to as a recessive disorder effect finding.

*Impact of Recessive Effect Finding*

Without additional data, it is unclear if this finding is important. Current standards for reporting the results of association studies are becoming more stringent. According to a statement by cancer researchers, "Single-gene studies will be considered increasingly less likely for publication when there are additional known biologically related and plausible genes or exposures to study. (p. 1985; Rebbeck et al., 2004)." In addition, publication priority will be given to variants with documented functional significance, such as laboratory-based studies showing that variants lead to differential function of the protein in question (Rebbeck et al., 2004). Therefore, publication of these findings would need to include results from other genes in the melanopsin-signaling pathway, or laboratory findings indicating that the P10L variant changes the function of the melanopsin protein.

*Lack of Associations: Summary*

Genotype and allele frequency at the I394T locus were not associated with case status (i.e., SAD or control) or seasonality as measured by the global seasonality score.

Genotypes and alleles at the D444G locus were monomorphic, meaning every participant had two copies of the major, or most common allele, and no individual had even one copy of the minor, less common allele. This is consistent with frequency data reported by other laboratories on the National Center for Biotechnology Reference Assembly on-line data base (http://www.ncbi.nlm.nih.gov/projects/SNP/), and suggests that this locus is not polymorphic, or very rarely so.

None of the SNPs were associated with degree of seasonality as measured by the global seasonality score on the Seasonal Pattern Assesment Questionnaire (SPAQ; Rosenthal et al., 1984a) among SAD participants. Six haplotypes in the melanopsin gene were identified and used in analyses testing an association between the melanopsin gene and case status (i.e., SAD diagnosis or no diagnosis controls). Haplotypes were also tested for an association with seasonality among SAD participants. None of the haplotypes identified were associated with SAD diagnosis or with degree of seasonality.

Using SAD participants, ancillary analyses were performed for two reasons, (1) to identify any potential symptom clusters associated with the melanopsing gene, and (2) to test the possibility that remission status after a trial of light therapy might be associated with the melanopsin gene. Tests using sub-scales and individual items on the SPAQ and Structured Interview Guide for the Hamilton Depression Rating Scale – SAD Version (SIGH-SAD; Williams et al., 1992b) showed that haplotype 1 was associated with low mood on dry days; haplotype 3 was associated with hypochondriasis and with no daily mood variation, and depersonalization; haplotype 4 was associated with psychomotor agitation; and haplotype 6 was associated with increased appetite, increased eating, and low mood in response to humid weather. Neither the P10L or I394T coding variant was associated with questionnaire items

or subscales, and none of the haplotypes were associated with subscales on the SPAQ or SIGH-SAD (i.e., typical depressive symptoms on the 21-item Hamilton Rating Scale for Depression or atypical depressive symptoms on the 8-item atypical subscale). There is no clear pattern to these findings, which are likely to be false positive results because of the number of analyses conducted. However, it is possible that having one or more copies of haplotype 6 may be associated with increased appetite and eating, given that eating behavior and appetite are partly under circadian control in humans (e.g., Hoogerwerf, 2006).

*Preliminary Analysis of Light Therapy Outcome*

This study also involved a preliminary test of association between remission with light therapy and the melanopsin gene. Light therapy outcome data were available for 45 individuals in the present study. No associations between P10L, I394T, or any haplotype and remission status after 2 weeks of light therapy were identified. There were two individuals treated with light that had the T/T P10L genotype, with one remitted and the other classified as not remitted. These data do not support a role for melanopsin variants in predicting light therapy remission rates.

*Possible Reasons for Lack of Findings*

The overall hypothesis of the present study, that melanopsin gene variants are associated with SAD, although theoretically compelling, was not supported by the results of the present study. Possible reasons for the lack of significant findings are that the melanopsin gene is not involved in SAD, important parts of the gene were not assayed (i.e., the newly reported isoform), or genes other than melanopsin are more important in conferring genetic risk for SAD, including those in the melanopsin signaling pathway (e.g., arrestin) and genes in other systems (e.g., neurotransmitters and circadian clock genes).

*Strengths of the Present Study*

One strength of the current study is that a very thorough analysis of the melanopsin gene was conducted, with fewer than 2K bases between markers. Dense maps for genetic analysis usually require at most 4K bases between markers; therefore, this analysis is even more dense. Because the genotypes at a nearby marker are highly correlated with each other, it is unlikely that the present study failed to assay important parts of the melanopsin gene. Therefore, it is most likely that genes other than melanopsin are responsible for the portion of risk for SAD that seems to be heritable. Other candidate genes for SAD are described below.

*Limitations*

*Recruitment Methods Differed*

An initial limitation of the study is that the control and SAD participants were recruited using different methods. The NIH healthy volunteer pool is a group of individuals who volunteer to be controls for many types of studies on a continual basis. However, the SAD participants were recruited through community advertising for the purpose of participating in SAD-specific studies at NIH, and were self-referred for treatment and/or research. This difference between recruitment methods for the comparison groups could have accounted for a difference between groups if one had been discovered in the present study. Hypothetically, a difference in genes could have been associated with a third factor other than SAD diagnosis, such as a factor associated with one of the recruitment strategies (i.e., willingness to volunteer for any research among controls vs. willingness only to volunteer for research on a specific issue among participants with SAD).

*Homogeneity of the Sample*

Another limitation is that both groups had very stringent inclusion and exclusion criteria, making any findings from the present study less generalizable to the population as a whole. Individuals in the SAD group had no personal history of another Axis I disorder, but research indicates that individuals with SAD may have comorbid ADHD, alcohol use disorders, generalized anxiety disorder, panic disorder, bulimia nervosa, late luteal phase dysphoric disorder, and chronic fatigue syndrome, although rates of comorbidity are not available (Lurie, Gawinski, Pierce, & Rousseau, 2006). Individuals in the control group had no personal or family history of Axis I disorders, and this is also unlikely to be a common finding. Ideally, for the present study, we would have information about how many potential SAD cases and control participants were excluded because they or a family member carried an Axis I disorder excluding them from participation. Despite a complete review of all records kept at NIH for the present study, data on number of individuals excluded was not discovered.

*Gender Differences Between Groups*

The SAD group was 70% female, but the control group was only 58% female, leaving open the possibility that gender has confounded our analyses. Although gender did not account for any variance in the logistic regression analyses, the difference between groups on the basis of gender is marginally significant ($p = 0.07$). It is unclear at this point if the difference in frequency of each gender has impacted the results, so future work should do one of two things: (1) test the effect of melanopsin variants in each group, stratified by gender, or (2) recruit a more gender-matched control group for future studies.

*Not All Candidate Genes Are Tested*

SAD is likely due to multiple risk factors, including multiple genes. Therefore, a limitation of the present study is that only one candidate gene for SAD was tested. Other genes could hypothetically be involved in SAD by increasing the threshold of light input necessary for euthymic functioning, or through mechanisms involved in neurotransmitter signaling pathways, the stress response system, or the circadian clock. Therefore, candidate genes for SAD not tested in the present study include those for molecular components of the light input pathway, neurotransmitter transporters and receptors, and circadian clock genes. Clock genes and neurotransmitter-related genes are in systems separate from the melanopsin signaling pathway, and should be included in any future work in this area.

*Future Directions*

Future directions for the present study should include other candidate genes for SAD and/or functional studies of melanopsin's P10L variation. As mentioned above, publication of association studies requires either laboratory-based findings that a given variant is functionally important, or must include other molecules involved in the same signaling pathway (Rebbeck et al., 2004). Molecules involved in the melanopsin-based signaling pathway described in the introduction are evaluated as possible candidate genes here, although other candidate genes (i.e., circadian clock genes, and neurotransmitter genes) are equally valid candidate genes for SAD, and could be investigated in future research on genetic risk factors in SAD. Molecular components of the non-visual light input pathway include the $G_q$ family of G proteins, phospholipase C (PLC), protein kinase C (PKC), inositol 1,4,5-triphosphate ($IP_3$), arrestin, and PACAP (Hannibal, 2006; Isoldi et al., 2005; Melyan et al., 2005; Panda et al., 2005; Qiu et al., 2005).

*Candidate Genes for SAD Other Than Melanopsin*

The G protein coupled to melanopsin is likely to be the G11 type member of the $G_q$ family (Panda et al., 2005), which is found in many different tissues in the body (Hubbard & Hepler, 2006), suggesting that any variations found in this protein would not be specific to the melanopsin signaling pathway or SAD. PLC-β4 binds to the $G_q$/G11α subunit, and PLC-β4 is found in the retina in mammals (Rhee, 2001). Phospholipase C (PLC) enzyme family members are involved in signal transduction by cleaving phospholipids into their component parts. PLC-β4 is found in multiple brain regions in rodents including olfactory cells, the thalamus, caudate putamen, and pituitary gland (Tanaka & Kondo, 1994). These data suggests that PLC-β4 is not specific to the melanopsin-based signaling pathway in humans either. The specific isozymes of PKC thought to be part of the melanopsin signaling pathway are beta 1 and 2 (Isoldi et al., 2005). These PKC isozymes (PRKCB1 and PRKCB2) are found in many tissues involved in multiple cellular functions and may be involved in disorders that are pervasive such as autism (Philippi et al., 2005), rather than more circumscribed disorders such as SAD. In cultured *Xenopus* cells expressing melanopsin, PKC phosphorylates at least four proteins (i.e., 43, 74, 90, and 134kDa), suggesting that the protein products or the process of phosphorylation are points in the signaling pathway that could be dysregulated in SAD (Isoldi et al., 2005). $IP_3$ receptors, thought to mediate the release of intracellular calcium in ipRGCs, are found in retinal tissue as well as smooth muscle cells. Therefore, it is likely that any functional variations in $IP_3$, PRKCB1, PKCB2, PLC-β4, or $G_q$/G11α that might impact melanopsin-based signaling would also have impacts on multiple other body systems, making these gene products unlikely candidates for a disorder like SAD.

*Candidate Genes For SAD: PKC*

In cultured *Xenopus* cells, PKC phosphorylates at least four proteins (i.e., 43-, 74-, 90-, and 134-kDa), suggesting that the protein products and/or the process of phosphorylation are points in the signaling pathway that could be disregulated in SAD (Isoldi et al., 2005). The proteins phosphorylated by PKC in the melanopsin signaling cascade are involved in melanasome dispersion in *Xenopus* cells, and it is currently unknown if these same proteins are phosphorylated in human ipRGCs, and if so, what their function is. Identifying the phosphorylation targets of PKCB1 and PKCB2 in ipRGCs may identify additional candidate genes for SAD, as it is possible that such light-activated pathway proteins are uniquely espressed in retinal cells, and have a unique function in ipRGCs.

*Candidate Genes For SAD: Arrestin*

As described above, arrestin is involved in the rhodopsin signaling cascade and a homologue of the rhodopsin arrestin may be present in ipRGCs. Arrestin binds to the photoreceptors in invertebrates (Kiselev & Subramaniam, 1997), and may bind human melanopsin at a site homologous to arrestin-binding sites in rhodopsin that are in the cytoplasmic loops of rhodopsin or phosphorylated residues on rhodopsin's tail (Ascano, Smith, Gregurick, & Robinson, 2006; Smith et al., 1999). Phosphorylation sites on the rhodopsin cytoplasmic tail are exposed when the chromophore changes shape, allowing arrestin to bind phosphorylated rhodopsin. If the sites at which arrestin binds rhodopsin are similar to sites in the cytoplasmic tail of melanopsin, these should be determined and sequenced in future studies. The current study assayed sites within 2K bases of possible arrestin-binding sites in melanopsin, but direct sequencing of this region would be necessary to rule out additional coding variations in this area.

*Candidate Genes for SAD: PACAP*

The neurotransmitter pituitary adenylate cyclase-activating polypeptide (PACAP), and glutamate are known to be neurotransmitters of the retino-hypothalamic track conveying light information from ipRGCs to the circadian clock (Hannibal, 2006). PACAP is found in the neural pathway from the retina to the SCN in the hypothalamus (Hannibal, 2006), in addition to other sites such as the pituitary. Similarly, glutamate is found in many pathways.

*Other Areas to Sequence in Melanopsin*

Motifs in melanopsin that are involved in light absorption are prime areas to consider when looking for potentially functional variants. Two sites in melanopsin are thought to interact with the chromophore, a Schiff's base lysine in the 7$^{th}$ transmembrane domain (K340) that binds the chromophore, and an invertebrate-like tyrosine "counterion" (Y146) in the 3rd transmembrane domain that assists in absorbing light (Provencio et al., 2000). These two supposed chromophore interaction sites in melanopsin are near two of the markers used in our analysis. The Schiff's base lysine is 54 amino acids from I394T, and the tyrosine counterion (Y146) is 136 amino acids from P10L. Each of these potentially critical amino acids (Y146 and K340) is proximal to a marker that was sequenced in our study. We expect that the sequences of polymorphisms this close are highly correlated, although the sequence of the Schiff's base and tyrosine counterion were not directly determined in the present study. Future studies could directly sequence these areas to determine if they are polymorphic, however, a review of on-line sequence databases revealed no known polymorphisms at these sites in melanopsin, although the coding variant rs7478092 is a polymorphism at amino acid position 334 or 323, depending on which isoform of melanopsin is being considered. Because rs7478092 could be as few as 6 amino acids upstream of K340,

this site could be sequenced to identify whether or not rs7478092 changes the Schiff's base lysine's ability to bind the chromophore.

*Newly Discovered Variants in Melanopsin Should be Sequenced*

Since the present study was designed, new variants in melanopsin have been identified and reported, including rs7478092 as described above. All new variants except one are synonymous changes, meaning the resultant amino acid sequence of the protein is not changed, as is the case with rs7478092. In one case, rs7901458, a new exon is introduced into the protein, such that a second isoform of melanopsin is created. An isoform is a version of a protein with a small difference, often caused by a single nucleotide polymorphism. It is currently unknown if this additional exon is functional or changes the function of melanopsin significantly. In future studies, the coding variant rs7901458, leading to the second melanopsin isoform, could be sequenced.

*Regulatory Genes are Candidate Genes*

Molecules interacting with the expression of melanopsin could be candidate genes for SAD, as well as molecules otherwise interacting with ipRGCs outside of the signaling and chromophore activities described above. Transcription factors have not yet been identified for melanopsin, but should be studied once identified. Melanopsin cells express AMPA receptors and $GABA_A$ receptors (Perez-Leon, Warren, Allen, Robinson, & Lane Brown, 2006). Therefore, receptors for these neurotransmitters are also possible targets for candidate gene analysis in SAD. These receptors likely are the targets of rod and cone inputs to melanopsin containing cells, as is the case in other primates (Dacey et al., 2005).

*Candidate Genes in Other Systems*

Other molecules involved in the neurotransmitter, hormonal, sleep, and HPA-axis/stress response systems may also be involved in SAD. For example, tryptophan hydroxylase (TPH), arylalkylamine N-acetyltranferase (AA-NAT), and hydroxyindole-O-methyltransferase (HIOMT) are enzymes that catalyze the transformation of tryptophan to serotonin and then into melatonin. AA-NAT, HIOMT, and TPH have been proposed as targets for pharmacological treatments for mood disorders because of the role of serotonin and melatonin in depression (Zheng & Cole, 2002). AA-NAT is the rate-limiting enzyme in the reaction transforming serotonin to melatonin, and may be involved in seasonally-dependent variations in serotonin levels because AA-NAT exhibits circadian and seasonal variations in expression (Diaz et al., 2003). Many other molecules interact with the various systems proposed to be involved in SAD etiology, making an exhaustive list of other candidate genes for SAD beyond the scope of the present study. However, it is clear that other candidate genes for SAD exist that have not yet been studied in SAD, and these genes may account for variance in the risk for SAD that is not accounted for by the known risk factors for SAD.

*Clinical Significance*

Despite the lack of significant findings, there are clear clinically-relevant reasons for pursuing this line of research. Finding a biological mechanism for the etiology of SAD could lead to more effective treatments for SAD that are curative (i.e., prevent recurrence after treatment is discontinued) as opposed to simply palliative (i.e., suppress symptoms as long as treatment is continued). Palliative treatments for SAD include light therapy and psychotropic medications that reduce the severity of symptoms during acute episodes. Light therapy, the

best available treatment for SAD, produces full remission in about half of individuals treated (Terman et al., 1990). Treating SAD with pharmacotherapy is also incomplete, with approximately half of individuals treated with fluoxetine achieving remission in a recent study (Lam et al., 2006). The best available indications of the potential success of cognitive-behavioral therapy for SAD indicates that this treatment is comparably efficacious to light therapy in acute SAD treatment (Rohan et al., in press), and may be superior to light therapy in prevention of future episodes (Rohan et al., 2007). However, cognitive-behavioral therapy is not designed to directly target a biological etiology for SAD. In addition, it is not known how many years the effects of CBT will last at this time. Developing durable treatments that target biological risk factors for SAD remains important.

*Genome Scan*

First and foremost in the pursuit of this line of research is to identify any other candidate genes for SAD and to determine their association with SAD diagnosis, seasonality, or symptom clusters that are characteristic to SAD. A technology that could be effective is a whole genome association study. Mapping arrays are high-density analyses that contain thousands of single nucleotide polymorphisms (SNPs) that cover the entire genome. These mapping arrays enable large-scale association studies with greater throughput and lower cost than technologies that existed when the present study was designed and in less time than previously possible. Specific mapping arrays that are commercially available may contain SNPs that could be used as markers for the genes mentioned above that are good candidate genes for SAD. Future studies could address whether or not currently available mapping arrays would be likely to test the identified candidate genes sufficiently.

References

Agumadu, C. O., Yousufi, S. M., Malik, I. S., Nguyen, M. C., Jackson, M. A., Soleymani, K., et al. (2004). Seasonal variation in mood in african american college students in the washington, d.C., metropolitan area. *American Journal of Psychiatry*, 161(6), 1084-1089.

Akiskal, H. S. (1985). Interaction of biologic and psychologic factors in the origin of depressive disorders. *Acta Psychiatrica Scandinavica. Supplementum*, 319, 131-139.

Ampollini, P., Marchesi, C., Signifredi, R., Chinaglia, E., Scardovi, F., Codeluppi, S., et al. (1999). Temperament and personality features in patients with major depression, panic disorder, and mixed condition. *Journal of Affective Disorders*, 52, 203-207.

Angold, A., Costello, E. J., & Worthman, C. M. (1998). Puberty and depression: The roles of age, pubertal status and pubertal timing. *Psychological Medicine*, 28(1), 51-61.

APA. (1987). *The diagnostic and statistical manual of mental disorders: Dsm-iii-r.* Washington, D.C.: American Psychiatric Association.

APA. (2000). *The diagnostic and statistical manual of mental disorders: Dsm-iv-tr* (4th ed. ed.). Washington, D.C.: American Psychiatric Association.

Ardlie, K. G., Lunetta, K. L., & Seielstad, M. (2002). Testing for population subdivision and association in four case-control studies. *American Journal of Human Genetics*, 71(2), 304-311.

Arias, B., Gutierrez, B., Pintor, L., Gasto, C., & Fananas, L. (2001). Variability in the 5-ht(2a) receptor gene is associated with seasonal pattern in major depression. *Molecular Psychiatry*, 6(2), 239-242.

Ascano, M. T., Smith, W. C., Gregurick, S. K., & Robinson, P. R. (2006). Arrestin residues involved in the functional binding of arrestin to phosphorylated, photolyzed rhodopsin. *Molecular Vision*, 12, 1516-1525.

Attia, J., Thakkinstian, A., & D'Este, C. (2003). Meta-analyses of molecular association studies: Methodologic lessons for genetic epidemiology. *Journal of Clinical Epidemiology*, 56(4), 297-303.

Avery, D., Dahl, K., Savage, M., Brengelmann, G., Larsen, L., Kenny, M., et al. (1997). Circadian temperature and cortisol rhythms during a constant routine are phase-delayed in hypersomnic winter depression. *Biological Psychiatry*, 41(11), 1109-1123.

Bagby, R. M., Ryder, A. G., Schuller, D. R., & Marshall, M. B. (2004). The hamilton depression rating scale: Has the gold standard become a lead weight? *American Journal of Psychiatry*, 161(12), 2163-2177.

Bagby, R. M., Schuller, D. R., Levitt, A. J., Joffe, R. T., & Harkness, K. L. (1996). Seasonal and non-seasonal depression and the five-factor model of personality. *Journal of Affective Disorders*, 38(2-3), 89-95.

Beck, A. T. (1967). *Depression: Clinical, experimental, and theoretical aspects.* New York: Hoeber.

Beck, A. T. (1976). *Cognitive therapy and emotional disorders.* New York: International Universities Press.

Beck, A. T., Rush, A. J., Shaw, B. F., & Emery, G. (1979). *Cognitive therapy of depression.* New York: Guilford Press.

Beck, A. T., Steer, R. A., & Brown, G. K. (1996). *Beck depression inventory - 2nd edition manual*. San Antonio, TX: The Psychological Corporation.

Berman, K., Lam, R. W., & Goldner, E. M. (1993). Eating attitudes in seasonal affective disorder and bulimia nervosa. *Journal of Affective Disorders*, 29(4), 219-225.

Bicakova-Rocher, A., Smolensky, M., Reinberg, A., & De Prins, J. (1985). Seasonal variations in socially and legally unacceptable sexual behaviour. *Chronobiology International*, 2(3), 203-208.

Blazer, D. G., Kessler, R. C., & Schwartz, M. S. (1998). Epidemiology of recurrent major and minor depression with a seasonal pattern: The national comorbidity survey. *British Journal of Psychiatry*, 172, 164-167.

Blouin, A., Blouin, J., Aubin, P., Carter, J., Goldstein, C., Boyer, H., et al. (1992). Seasonal patterns of bulimia nervosa. *American Journal of Psychiatry*, 149(1), 73-81.

Bunney, W. E., & Bunney, B. G. (2000). Molecular clock genes in man and lower animals: Possible implications for circadian abnormalities in depression. *Neuropsychopharmacology*, 22(4), 335-345.

Campbell, C. D., Ogburn, E. L., Lunetta, K. L., Lyon, H. N., Freedman, M. L., Groop, L. C., et al. (2005). Demonstrating stratification in a european american population. *Nature Genetics*, 37(8), 868-872.

Cardon, L. R., & Bell, J. I. (2001). Association study designs for complex diseases. *Nature Genetics*, 2, 91-99.

Carlson, C. S., Eberle, M. A., Rieder, M. J., Yi, Q., Kruglyak, L., & Nickerson, D. A. (2004). Selecting a maximally informative set of single-nucleotide polymorphisms for association analyses using linkage disequilibrium. *American Journal of Human Genetics*, 74(1), 106-120.

Carskadon, M. A., & Acebo, C. (1993). Parental reports of seasonal mood and behavior changes in children. *Journal of the American Academy of Child and Adolescent Psychiatry*, 32(2), 264-269.

Caspi, A., Sugden, K., Moffitt, T. E., Taylor, A., Craig, I. W., Harrington, H., et al. (2003). Influence of life stress on depression: Moderation by a polymorphism in the 5-htt gene. *Science*, 301, 386-389.

Cavali-Sforza, L. L., Menozzi, P., & Piazza, A. (1994). *The history and geography of human genes*. Princeton, NJ: Princeton University Press.

Chalmers, I., Enkin, M., & Keirse, M. (1989). *Effective care in pregnancy and childbirth.* New York: Oxford University Press.

Chen, H. S., Zhu, X., Zhao, H., & Zhang, S. (2003). Qualitative semi-parametric test for genetic associations in case-control designs under structured populations. *Annals of Human Genetics*, 67(Pt 3), 250-264.

Cheverud, J. M. (2001). A simple correction for multiple comparisons in interval mapping genome scans. *Heredity*, 87(Pt 1), 52-58.

Clark, A. G. (2004). The role of haplotypes in candidate gene studies. *Genetic Epidemiology*, 27(4), 321-333.

Clark, L. A., Watson, D., & Mineka, S. (1994). Temperament, personality, and the mood and anxiety disorders. *Journal of Abnormal Psychology*, 103(1), 103-116.

Clark, V. J., & Dean, M. (2004). Haplotype structure and linkage disequilibrium in chemokine and chemokine receptor genes. *Hum Genomics*, 1(4), 255-273.

Cloninger, C. R., Bayon, C., & Svrakic, D. M. (1998a). Measurement of temperament and character in mood disorders: A model of fundamental states as personality types. *Journal of Affective Disorders*, 51, 21-32.

Cloninger, C. R., Van Eerdewegh, P., Goate, A., Edenberg, H. J., Blangero, J., Hesselbrock, V., et al. (1998b). Anxiety proneness linked to epistatic loci in genome scan of human personality traits. *American Journal of Medical Genetics*, 81(4), 313-317.

Cohen, J. (1988). *Statistical power and analysis for the behavioral sciences* (2nd ed.). Hillsdale, NJ: Erlbaum Associates.

Cordell, H. J., & Clayton, D. G. (2005). Genetic association studies. *The Lancet*, 366, 1121-1131.

Costa, P. T., & McCrae, R. R. (1992). *Revised neo personality inventory (neo-pi-r) and neo five inventory (neo-ffi) professional manual.* Odessa, FL: Psychological Assessment Resources.

Crawford, D. C., Bhangale, T., Li, N., Hellenthal, G., Rieder, M. J., Nickerson, D. A., et al. (2004). Evidence for substantial fine-scale variation in recombination rates across the human genome. *Nature Genetics*, 36(7), 700-706.

Curran, J. M., Buckleton, J. S., & Triggs, C. M. (2003). What is the magnitude of the subpopulation effect? *Forensic Science International*, 135(1), 1-8.

Czeisler, C. A., Kronauer, R. E., Allan, J. S., Duffy, J. F., Jewett, M. E., Brown, E. N., et al. (1989). Bright light induction of strong (type 0) resetting of the human circadian pacemaker. *Science*, 244(4910), 1328-1333.

Dacey, D. M., Liao, H. W., Peterson, B. B., Robinson, F. R., Smith, V. C., Pokorny, J., et al. (2005). Melanopsin-expressing ganglion cells in primate retina signal colour and irradiance and project to the lgn. *Nature*, 433(7027), 749-754.

Dahl, K., Avery, D. H., Lewy, A. J., Savage, M. V., Brengelmann, G. L., Larsen, L. H., et al. (1993). Dim light melatonin onset and circadian temperature during a constant routine in hypersomnic winter depression. *Acta Psychiatrica Scandinavica*, 88(1), 60-66.

Daly, A. K. (2003). Candidate gene case-control studies. *Pharmacogenomics*, 4(2), 127-139.

Devlin, B., & Roeder, K. (1999). Genomic control for association studies. *Biometrics*, 55, 997-1004.

Diaz, E., Garidou, M. L., Dardente, H., Salingre, A., Pevet, P., & Simonneaux, V. (2003). Expression and regulation of icer mrna in the syrian hamster pineal gland. *Brain Research. Molecular Brain Research*, 112(1-2), 163-169.

Dikeos, D. G., Papadimitriou, G. N., Souery, D., Del-Favero, J., Massat, I., Blackwood, D., et al. (2006). Lack of genetic association between the phospholipase a2 gene and bipolar mood disorder in a european multicentre case-control study. *Psychiatric Genetics*, 16(4), 169-171.

Duman, R. S., Heninger, G. R., & Nestler, E. J. (1997). A molecular and cellular theory of depression. *Archives of General Psychiatry*, 54(7), 597-606.

Duncan, W. C., Jr. (1996). Circadian rhythms and the pharmacology of affective illness. *Pharmacology & Therapeutics*, 71(3), 253-312.

Dunlap, J. C., Loros, J. J., & DeCoursey, P. J. (2004). *Chronobiology: Biological timekeeping*. Sunderland, MA: Sinauer Associates.

Ehlers, C. L., Frank, E., & Kupfer, D. J. (1988). Social zeitgebers and biological rhythms. A unified approach to understanding the etiology of depression. *Archives of General Psychiatry*, 45(10), 948-952.

Enoch, M. A., Goldman, D., Barnett, R., Sher, L., Mazzanti, C. M., & Rosenthal, N. E. (1999). Association between seasonal affective disorder and the 5-ht2a promoter polymorphism, -1438g/a. *Molecular Psychiatry*, 4(1), 89-92.

Fallin, D., Cohen, A., Essioux, L., Chumakov, I., Blumenfeld, M., Cohen, D., et al. (2001). Genetic analysis of case/control data using estimated haplotype frequencies: Application to apoe locus variation and alzheimer's disease. *Genome Research*, 11(1), 143-151.

Fennig, S., Craig, T., Lavelle, J., Kovasznay, B., & Bromet, E. J. (1994). Best-estimate versus structured interview-based diagnosis in first-admission psychosis. *Comprehensive Psychiatry*, 35(5), 341-348.

First, M. B., Spitzer, R. L., Gibbon, M., & Williams, J. B. W. (1995). *Structured clinical interview for dsm-iv axis i disorders (scid - clinician version)*. New York, NY: New York State Psychiatric Institute Biometrics Research Department.

Fornari, V. M., Braun, D. L., Sunday, S. R., Sandberg, D. E., Matthews, M., Chen, I. L., et al. (1994). Seasonal patterns in eating disorder subgroups. *Comprehensive Psychiatry*, 35(6), 450-456.

Freedman, M. L., Reich, D., Penney, K. L., McDonald, G. J., Mignault, A. A., Patterson, N., et al. (2004). Assessing the impact of population stratification on genetic association studies. *Nature Genetics*, 36(4), 388-393.

Gelehrter, T. D., Collins, F. S., & Ginsburg, D. (Eds.). (1998). Principles of medical genetics (2nd ed.). Baltimore, Maryland: Williams & Wilkins.

Golden, R. N., Gaynes, B. N., Ekstrom, R. D., Hamer, R. M., Jacobsen, F. M., Suppes, T., et al. (2005). The efficacy of light therapy in the treatment of mood disorders: A review and meta-analysis of the evidence. *American Journal of Psychiatry*, 162(4), 656-662.

Gooley, J. J., Lu, J., Fischer, D., & Saper, C. B. (2003). A broad role for melanopsin in nonvisual photoreception. *Journal of Neuroscience*, 23(18), 7093-7106.

Gordon, T., Keel, J., Hardin, T. A., & Rosenthal, N. E. (1999). Seasonal mood change and neuroticism: The same construct? *Comprehensive Psychiatry*, 40(6), 415-417.

Gorroochurn, P., Hodge, S. E., Heiman, G., & Greenberg, D. A. (2004). Effect of population stratification on case-control association studies. Ii. False-positive rates and their limiting behavior as number of subpopulations increases. *Human Heredity*, 58(1), 40-48.

Graw, P., Recker, S., Sand, L., Krauchi, K., & Wirz-Justice, A. (1999). Winter and summer outdoor light exposure in women with and without seasonal affective disorder. *Journal of Affective Disorders*, 56(2-3), 163-169.

Greenberg, B. D., Li, Q., Lucas, F. R., Hu, S., Sirota, L. A., Benjamin, J., et al. (2000). Association between the serotonin transporter promoter polymorphism and personality traits in a primarily female population sample. *American Journal of Medical Genetics*, 96(2), 202-216.

Hannibal, J. (2006). Roles of pacap-containing retinal ganglion cells in circadian timing. *International Review of Cytology*, 251, 1-39.

Harrison, P. J. (2002). The neuropathology of primary mood disorder. *Brain*, 125, 1428-1449.

Hasin, D. S., Goodwin, R. D., Stinson, F. S., & Grant, B. F. (2005). Epidemiology of major depressive disorder: Results from the national epidemiologic survey on alcoholism and related conditions. *Archives of General Psychiatry*, 62(10), 1097-1106.

Hattar, S., Liao, H., Takao, M., Berson, D., & Yau, K. (2002). Melanopsin-containing retinal ganglion cells: Architecture, projections, and intrinsic photosensitivity. *Science*, 295(5557), 1065-1070.

Hayley, S., Poulter, M. O., Merali, Z., & Anisman, H. (2005). The pathogenesis of clinical depression: Stressor- and cytokine-induced alterations of neuroplasticity. *Neuroscience*, 135(3), 659-678.

Hebert, M., Dumont, M., & Lachapelle, P. (2002). Electrophysiological evidence suggesting a seasonal modulation of retinal sensitivity in subsyndromal winter depression. *Journal of Affective Disorders*, 68(2-3), p191-202.

Hirschhorn, J. N., Lohmueller, K., Byrne, E., & Hirschhorn, K. (2002). A comprehensive review of genetic association studies. *Genet Med*, 4(2), 45-61.

Hoggart, C. J., Parra, E. J., Shriver, M. D., Bonilla, C., Kittles, R. A., Clayton, D. G., et al. (2003). Control of confounding of genetic associations in stratified populations. *American Journal of Human Genetics*, 72(6), 1492-1504.

Hoh, J., Wille, A., & Ott, J. (2001). Trimming, weighting, and grouping snps in human case-control association studies. *Genome Research*, 11(12), 2115-2119.

Hoogerwerf, W. A. (2006). Biologic clocks and the gut. *Curr Gastroenterol Rep*, 8(5), 353-359.

Hsieh, F. Y. (1989). Sample size tables for logistic regression. *Statistics in Medicine*, 8, 795-802.

Hubbard, K. B., & Hepler, J. R. (2006). Cell signalling diversity of the gqalpha family of heterotrimeric g proteins. *Cellular Signalling*, 18(2), 135-150.

Hutchison, K. E., Stallings, M., McGeary, J., & Bryan, A. (2004). Population stratification in the candidate gene study: Fatal threat or red herring? *Psychological Bulletin*, 130(1), 66-79.

Insel, T. R., & Winslow, J. T. (1998). Serotonin and neuropeptides in affiliative behaviors. *Biological Psychiatry*, 44(3), 207-219.

Ioannidis, J. P. (2007). Non-replication and inconsistency in the genome-wide association setting. *Human Heredity*, 64(4), 203-213.

Ioannidis, J. P., Ntzani, E. E., Trikalinos, T. A., & Contopoulous-ioannidis, D. G. (2001). Replication validity of genetic association studies. *Nature Genetics*, 29(3), 306-309.

Isoldi, M. C., Rollag, M. D., Castrucci, A. M., & Provencio, I. (2005). Rhabdomeric phototransduction initiated by the vertebrate photopigment melanopsin. *Proceedings of the National Academy of Sciences of the United States of America*, 102(4), 1217-1221.

Jain, U., Blais, M. A., Otto, M. W., Hirshfeld, D. R., & Sachs, G. S. (1999). Five-factor personality traits in patients with seasonal depression: Treatment effects and comparisons with bipolar patients. *Journal of Affective Disorders*, 55(1), 51-54.

Jang, K. L., Lam, R. W., Harris, J. A., Vernon, P. A., & Livesley, W. J. (1998). Seasonal mood change and personality: An investigation of genetic co-morbidity. *Psychiatry Research*, Vol 78(1-2), 1-7.

Jang, K. L., Lam, R. W., Livesley, W. J., & Vernon, P. A. (1997). Gender differences in the heritability of seasonal mood change. *Psychiatry Research*, Vol 70(3), 145-154.

Joffe, R. T., Bagby, R. M., Levitt, A. J., Regan, J. J., & Parker, J. D. A. (1993). The tridimensional personality questionnaire in major depression. *American Journal of Psychiatry*, 150, 959-960.

Johansson, C., Smedh, C., Partonen, T., Pekkarinen, P., Paunio, T., Ekholm, J., et al. (2001). Seasonal affective disorder and serotonin-related polymorphisms. *Neurobiology of Disease*, 8(2), 351-357.

Johansson, C., Willeit, M., Levitan, R., Partonen, T., Smedh, C., Del Favero, J., et al. (2003a). The serotonin transporter promoter repeat length polymorphism, seasonal affective disorder and seasonality. *Psychological Medicine*, 33(5), 785-792.

Johansson, C., Willeit, M., Smedh, C., Ekholm, J., Paunio, T., Kieseppa, T., et al. (2003b). Circadian clock-related polymorphisms in seasonal affective disorder and their relevance to diurnal preference. *Neuropsychopharmacology*, 28(4), p734-739.

Joyner, K., & Udry, J. R. (2000). You don't bring me anything but down: Adolescent romance and depression. *Journal of Health and Social Behavior*, 41(4), 369-391.

Kalia, M. (2005). Neurobiological basis of depression: An update. *Metabolism: Clinical and Experimental*, 54(5 Suppl 1), 24-27.

Kandel, E. R., Schwartz, J. H., & Jessell, T. M. (2000). *Principles of neural science*: Appleton & Lange.

Kasper, S., Wehr, T. A., Bartko, J. J., Gaist, P. A., & Rosenthal, N. E. (1989). Epidemiological findings of seasonal changes in mood and behavior. A telephone

survey of montgomery county, maryland. *Archives of General Psychiatry*, 46(9), 823-833.

Kempermann, G., & Kronenberg, G. (2003). Depressed new neurons--adult hippocampal neurogenesis and a cellular plasticity hypothesis of major depression. *Biological Psychiatry*, 54(5), 499-503.

Kendler, K. S., Gardner, C. O., & Prescott, C. A. (2003). Personality and the experience of environmental adversity. *Psychological Medicine*, 33(7), 1193-1202.

Kessler, R. C. (2002). Epidemiology of depression. In I. H. Gotlib & C. L. Hammen (Eds.), Handbook of depression (pp. 23-42). New York: The Guilford Press.

Kessler, R. C., Berglund, P., Demler, O., Jin, R., Koretz, D., Merikangas, K. R., et al. (2003). The epidemiology of major depressive disorder: Results from the national comorbidity survey replication (ncs-r). *JAMA*, 289(23), 3095-3105.

Khalsa, S. B. S., Jewett, M. E., Duffy, J. F., & Czeisler, C. A. (2000). The timing of the human circadian clock is accurately represented by the core body temperature rhythm following phase shifts to a three-cycle light stimulus near the critical zone. *Journal of Biological Rhythms*, 15(6), 524-530.

Khlat, M., Cazes, M. H., Genin, E., & Guiguet, M. (2004). Robustness of case-control studies of genetic factors to population stratification: Magnitude of bias and type i error. *Cancer Epidemiology, Biomarkers and Prevention*, 13(10), 1660-1664.

Kiselev, A., & Subramaniam, S. (1997). Studies of rh1 metarhodopsin stabilization in wild-type drosophila and in mutants lacking one or both arrestins. *Biochemistry*, 36(8), 2188-2196.

Kittles, R. A., Chen, W., Panguluri, R. K., Ahaghotu, C., Jackson, A., Adebamowo, C. A., et al. (2002). Cyp3a4-v and prostate cancer in african americans: Causal or confounding association because of population stratification? *Human Genetics*, 110(6), 553-560.

Klein, D. N., Durbin, C. E., Shankman, S. A., & Santiago, N. J. (2002). Depression and personality. In I. H. Gotlib & C. L. Hammen (Eds.), Handbook of depression (pp. 115-140). New York: The Guilford Press.

Knowler, W. C., Williams, R. C., Pettitt, D. J., & Steinberg, A. G. (1988). Gm3;5,13,14 and type 2 diabetes mellitus: An association in american indians with genetic admixture. *American Journal of Human Genetics*, 43(4), 520-526.

Koller, D. L., Peacock, M., Lai, D., Foroud, T., & Econs, M. J. (2004). False positive rates in association studies as a function of degree of stratification. *Journal of Bone and Mineral Research*, 19(8), 1291-1295.

Kranzler, H. R., Kadden, R. M., Babor, T. F., Tennen, H., & Rounsaville, B. J. (1996). Validity of the scid in substance abuse patients. *Addiction*, 91(6), 859-868.

Kranzler, H. R., Kadden, R. M., Burleson, J. A., Babor, T. F., Apter, A., & Rounsaville, B. J. (1995). Validity of psychiatric diagnoses in patients with substance use disorders: Is the interview more important than the interviewer? *Comprehensive Psychiatry*, 36(4), 278-288.

Krauchi, K., Reich, S., & Wirz-Justice, A. (1997). Eating style in seasonal affective disorder: Who will gain weight in winter? *Comprehensive Psychiatry*, 38(2), 80-87.

Krueger, R. F. (1999). Personality traits in late adolescence predict mental disorders in early adulthood: A prospective-epidemiological study. *Journal of Personality*, 67(1), 39-65.

Kumbalasiri, T., Rollag, M. D., Isoldi, M., de Lauro Castrucci, A. M., & Provencio, I. (2006). Melanopsin triggers the release of internal calcium stores in response to light. *Photochemistry and Photobiology.*

Lam, R. W., Beattie, C. W., Buchanan, A., Remick, R. A., & Zis, A. P. (1991). Low electrooculographic ratios in patients with seasonal affective disorder. *American Journal of Psychiatry*, 148(11), 1526-1529.

Lam, R. W., Buchanan, A., & Remick, R. A. (1989). Seasonal affective disorder - a canadian sample. *Annals of clinical psychiatry*, 1, 241-245.

Lam, R. W., Solyom, L., & Tompkins, A. (1991). Seasonal mood symptoms in bulimia nervosa and seasonal affective disorder. *Comprehensive Psychiatry*, 32(6), 552-558.

Lam, R. W., Tam, E. M., Yatham, L. N., Shiah, I. S., & Zis, A. P. (2001). Seasonal depression: The dual vulnerability hypothesis revisited. *Journal of Affective Disorders*, 63(1-3), 123-132.

Lam, R. W., Zis, A. P., Grewal, A., Delgado, P. L., Charney, D. S., & Krystal, J. H. (1996). Effects of rapid tryptophan depletion in patients with seasonal affective disorder in remission after light therapy. *Archives of General Psychiatry*, 53(1), 41-44.

Lambert, G., Reid, C., Kaye, D., Jennings, G., & Esler, M. (2002). Effect of sunlight and season on serotonin turnover in the brain. *Lancet*, 360(9348), p1840-1842.

Lander, E. S., & Botstein, D. (1989). Mapping mendelian factors underlying quantitative traits using rflp linkage maps. *Genetics*, 121(1), 185-199.

Lee, J. E., Choi, J. H., Lee, J. H., & Lee, M. G. (2005). Gene snps and mutations in clinical genetic testing: Haplotype-based testing and analysis. *Mutation Research*, 573(1-2), 195-204.

Lee, T. M. C., Chan, C. C. H., Paterson, J. G., Janzen, H. L., & Blashko, C. A. (1997). Spectral properties of phototherapy for seasonal affective disorder: A meta-analysis. *Acta Psychiatrica Scandinavica*, Vol 96(2), 117-121.

Levinson, D. F. (2005). The genetics of depression: A review. *Biological Psychiatry*, in press.

Levitan, R. D., Masellis, M., Basile, V. S., Lam, R. W., Kaplan, A. S., Davis, C., et al. (2004a). The dopamine-4 receptor gene associated with binge eating and weight gain in women with seasonal affective disorder: An evolutionary perspective. *Biological Psychiatry*, 56(9), 665-669.

Levitan, R. D., Masellis, M., Lam, R. W., Muglia, P., Basile, V. S., Jain, U., et al. (2004b). Childhood inattention and dysphoria and adult obesity associated with the dopamine d4 receptor gene in overeating women with seasonal affective disorder. *Neuropsychopharmacology*, 29(1), 179-186.

Levitt, A. J., Joffe, R. T., Brecher, D., & MacDonald, C. (1993). Anxiety disorders and anxiety symptoms in a clinic sample of seasonal and non-seasonal depressives. *Journal of Affective Disorders*, 28(1), 51-56.

Lewinsohn, P. M. (1974). A behavioral approach to depression. In R. J. Friedman & M. M. Katz (Eds.), The psychology of depression: Contemporary theory and research (pp. 157-178). Washington, DC: John Wiley & Sons.

Lewinsohn, P. M. (1985). An integrative theory of depression. In S. Reiss & R. R. Bootzin (Eds.), Theoretical issues in behavior therapy. (pp. 331-359). Orlando: Academic Press.

Lewy, A. J., Sack, R. L., Singer, C. M., & White, D. M. (1987). The phase shift hypothesis for bright light's therapeutic mechanism of action: Theoretical considerations and experimental evidence. *Psychopharmacology Bulletin*, 23(3), 349-353.

Lipsey, M. W., & Wilson, D. B. (2001). *Practical meta-analysis*. Thousand Oaks, CA: Sage Publications.

Long, A. D., & Langley, C. H. (1999). The power of association studies to detect the contribution of candidate genetic loci to variation in complex traits. *Genome Research*, 9(8), 720-731.

Lopez, J. F., Liberzon, I., Vazquez, D. M., Young, E. A., & Watson, S. J. (1999). Serotonin 1a receptor messenger rna regulation in the hippocampus after acute stress. *Biological Psychiatry*, 45(7), 934-937.

Lucas, R. J., Hattar, S., Takao, M., Berson, D. M., Foster, R. G., & Yau, K. W. (2003). Diminished pupillary light reflex at high irradiances in melanopsin-knockout mice. *Science*, 299(5604), 245-247.

Lurie, S. J., Gawinski, B., Pierce, D., & Rousseau, S. J. (2006). Seasonal affective disorder. *American Family Physician*, 74(9), 1521-1524.

Madden, P. A., Heath, A. C., Rosenthal, N. E., & Martin, N. G. (1996). Seasonal changes in mood and behavior. The role of genetic factors. *Archives of General Psychiatry*, 53(1), 47-55.

Maeno, N., Kusunoki, K., Kitajima, T., Iwata, N., Ono, Y., Hashimoto, S., et al. (2005). Personality of seasonal affective disorder analyzed by tri-dimensional personality questionnaire. *Journal of Affective Disorders*, 85(3), 267-273.

Maes, M., & Meltzer, H. Y. (1995). The serotonin hypothesis of major depression. In F. E. Bloom & D. J. Kupfer (Eds.), Psychopharmacology: The fourth generation of progress (pp. 933-944). New York: Raven Press.

Magnusson, A. (2000). An overview of epidemiological studies on seasonal affective disorder. *Acta Psychiatrica Scandinavica*, 101(3), 176-184.

Magnusson, A., & Boivin, D. (2003). Seasonal affective disorder: An overview. *Chronobiology International*, 20(2), 189-207.

Magnusson, A., & Partonen, T. (2005). The diagnosis, symptomatology, and epidemiology of seasonal affective disorder. *CNS Spectr*, 10(8), 625-634; quiz 621-614.

Manji, H. K., & Duman, R. S. (2001). Impairments of neuroplasticity and cellular resilience in severe mood disorders: Implications for the development of novel therapeutics. *Psychopharmacology Bulletin*, 35(2), 5-49.

Marriott, P. F., Armstrong, S. M., & Hay, D. (1994a). Spaq reliability in an australian twin sample. *Light Treatment and Biological Rhythms*, 5(3), 32.

Marriott, P. F., Greenwood, K. M., & Armstrong, S. M. (1994b). Seasonality in panic disorder. *Journal of Affective Disorders*, 31(2), 75-80.

Meesters, Y., Jansen, J., Beersma, D., Bouhuys, A., & van den Hoofdakker, R. (1995). Light therapy for seasonal affective disorder. The effects of timing. *British Journal of Psychiatry*, 166(5), 607-612.

Meesters, Y., & Jansen, J. H. (1993). Assessing atypical seasonal affective disorder complaints by means of self-rating. *Acta Psychiatrica Scandinavica*, 88(5), 361-363.

Melyan, Z., Tarttelin, E. E., Bellingham, J., Lucas, R. J., & Hankins, M. W. (2005). Addition of human melanopsin renders mammalian cells photoresponsive. *Nature*, 433(7027), 741-745.

Mersch, P., Middendorp, H., Bouhuys, A., Beersma, D., & van den Hoofdakker, R. (1999). Seasonal affective disorder and latitude: A review of the literature. *Journal of Affective Disorders*, 53(1), 35-48.

Mersch, P. P., Vastenburg, N. C., Meesters, Y., Bouhuys, A. L., Beersma, D. G., van den Hoofdakker, R. H., et al. (2004a). The reliability and validity of the seasonal pattern assessment questionnaire: A comparison between patient groups. *Journal of Affective Disorders*, 80(2-3), 209-219.

Mersch, P. P., Vastenburg, N. C., Meesters, Y., Bouhuys, A. L., Beersma, D. G., van den Hoofdakker, R. H., et al. (2004b). The reliability and validity of the seasonal pattern assessment questionnaire: A comparison between patient groups. *Journal of Affective Disorders*, 80(2-3), 209-219.

Molin, J., Mellerup, E., Bolwig, T., Scheike, T., & Dam, H. (1996). The influence of climate on development of winter depression. *Journal of Affective Disorders*, 37(2-3), 151-155.

Montell, C. (2005). Trp channels in drosophila photoreceptor cells. *J Physiol*, 567(Pt 1), 45-51.

Morin, L. P., Blanchard, J. H., & Provencio, I. (2003). Retinal ganglion cell projections to the hamster suprachiasmatic nucleus, intergeniculate leaflet, and visual midbrain: Bifurcation and melanopsin immunoreactivity. *Journal of Comparative Neurology*, 465(3), 401-416.

Morton, N. E., & Collins, A. (1998). Tests and estimates of allelic association in complex inheritance. *Proceedings of the National Academy of Sciences of the United States of America*, 95(19), 11389-11393.

Moscovitch, A., Blashko, C. A., Eagles, J. M., Darcourt, G., Thompson, C., Kasper, S., et al. (2004). A placebo-controlled study of sertraline in the treatment of outpatients with seasonal affective disorder. *Psychopharmacology*, 171(4), 390-397.

Murphy, D., Murphy, D., Abbas, M., Palazidou, E., Binnie, C., Arendt, J., et al. (1993). Seasonal affective disorder: Response to light as measured by electroencephalogram, melatonin suppression, and cerebral blood flow. *British Journal of Psychiatry*, 163, 327-331, 335-327.

Neumeister, A., Turner, E. H., Matthews, J. R., Postolache, T. T., Barnett, R. L., Rauh, M., et al. (1998). Effects of tryptophan depletion vs catecholamine depletion in patients with seasonal affective disorder in remission with light therapy. *Archives of General Psychiatry*, 55(6), 524-530.

Newton-Cheh, C., & Hirschhorn, J. N. (2005). Genetic association studies of complex traits: Design and analysis issues. *Mutation Research*, 573(1-2), 54-69.

Niu, T., Qin, Z. S., Xu, X., & Liu, J. S. (2002). Bayesian haplotype inference for multiple linked single-nucleotide polymorphisms. *American Journal of Human Genetics*, 70(1), 157-169.

Nolen-Hoeksema, S. (1987). Sex differences in unipolar depression: Evidence and theory. *Psychological Bulletin*, 101, 259-282.

Oren, D. A., Brainard, G. C., Johnston, S. H., Joseph-Vanderpool, J. R., Sorek, E., & Rosenthal, N. E. (1991). Treatment of seasonal affective disorder with green light and red light. *American Journal of Psychiatry*, 148(4), 509-511.

Ozaki, N., Rosenthal, N. E., Myers, F., Schwartz, P. J., & Oren, D. A. (1995). Effects of season on electro-oculographic ratio in winter seasonal affective disorder. *Psychiatry Research*, 59(1-2), 151-155.

Ozaki, N., Rosenthal, N. E., Pesonen, U., Lappalainen, J., Feldman-Naim, S., Schwartz, P. J., et al. (1996). Two naturally occurring amino acid substitutions of the 5-ht2a receptor: Similar prevalence in patients with seasonal affective disorder and controls. *Biological Psychiatry*, 40(12), 1267-1272.

Panda, S., Nayak, S. K., Campo, B., Walker, J. R., Hogenesch, J. B., & Jegla, T. (2005). Illumination of the melanopsin signaling pathway. *Science*, 307(5709), 600-604.

Panda, S., Provencio, I., Tu, D. C., Pires, S. S., Rollag, M. D., Castrucci, A. M., et al. (2003). Melanopsin is required for non-image-forming photic responses in blind mice. *Science*, 301, 525-527.

Panda, S., Sato, T. K., Castrucci, A. M., Rollag, M. D., De Grip, W. J., Hogenesch, J. B., et al. (2002). Melanopsin (opn4) requirement for normal light-induced circadian phase shifting. *Science*, 298(5601), 2213-2216.

Pankow, J. S., Province, M. A., Hunt, S. C., & Arnett, D. K. (2002). Regarding "Testing for population subdivision and association in four case-control studies". *American Journal of Human Genetics*, 71(6), 1478-1480.

Paykel, E. S. (1979). Reading about...Depression--clinical aspects. *British Journal of Psychiatry*, 134, 211-213.

Perez-Leon, J. A., Warren, E. J., Allen, C. N., Robinson, D. W., & Lane Brown, R. (2006). Synaptic inputs to retinal ganglion cells that set the circadian clock. *European Journal of Neuroscience*, 24(4), 1117-1123.

Philippi, A., Roschmann, E., Tores, F., Lindenbaum, P., Benajou, A., Germain-Leclerc, L., et al. (2005). Haplotypes in the gene encoding protein kinase c-beta (prkcb1) on chromosome 16 are associated with autism. *Molecular Psychiatry*, 10(10), 950-960.

Plomin, R., DeFries, J. C., Craig, I. W., & McGuffin, P. (2003). Behavioral genetics. In R. Plomin, J. C. DeFries, I. W. Craig & P. McGuffin (Eds.), Behavioral genetics in the postgenomic era. Washington, DC: American Psychological Association.

Pritchard, J. K., & Donnelly, P. (2001). Case-control studies of association in structured or admixed populations. *Theoretical Population Biology*, 60(3), 227-237.

Pritchard, J. K., & Rosenberg, N. A. (1999). Use of unlinked genetic markers to detect population stratification in association studies. *American Journal of Human Genetics*, 65, 220-228.

Provencio, I. (2004). Chronobiology. In Comprehensive textbook of psychiatry (8th ed.).

Provencio, I., Jiang, G., De Grip, W. J., Hayes, W. P., & Rollag, M. D. (1998). Melanopsin: An opsin in melanophores, brain, and eye. *Proceedings of the National Academy of Sciences of the United States of America*, 95(1), 340-345.

Provencio, I., Rodriguez, I. R., Jiang, G., Hayes, W. P., Moreira, E. F., & Rollag, M. D. (2000). A novel human opsin in the inner retina. *Journal of Neuroscience*, 20(2), 600-605.

Provencio, I., Rollag, M. D., & Castrucci, A. M. (2002). Photoreceptive net in the mammalian retina. This mesh of cells may explain how some blind mice can still tell day from night. *Nature*, 415(6871), 493.

Qiu, X., Kumbalasiri, T., Carlson, S. M., Wong, K. Y., Krishna, V., Provencio, I., et al. (2005). Induction of photosensitivity by heterologous expression of melanopsin. *Nature*, 433(7027), 745-749.

Raheja, S. K., King, E. A., & Thompson, C. (1996). The seasonal pattern assessment questionnaire for identifying seasonal affective disorders. *Journal of Affective Disorders*, 41(3), 193-199.

Ramirez Basco, M., Bostic, J. Q., Davies, D., Rush, A. J., Witte, B., Hendrickse, W., et al. (2000). Methods to improve diagnostic accuracy in a community mental health setting. *American Journal of Psychiatry*, 157(10), 1599-1605.

Rebbeck, T. R., Martinez, M. E., Sellers, T. A., Shields, P. G., Wild, C. P., & Potter, J. D. (2004). Genetic variation and cancer: Improving the environment for publication of association studies. *Cancer Epidemiology, Biomarkers and Prevention*, 13(12), 1985-1986.

Reid, S., Towell, A. D., & Golding, J. F. (2000). Seasonality, social zeitgebers and mood variability in entrainment of mood. Implications for seasonal affective disorder. *Journal of Affective Disorders*, 59(1), 47-54.

Rhee, S. G. (2001). Regulation of phosphoinositide-specific phospholipase c. *Annual Review of Biochemistry*, 70, 281-312.

Richter, J., Eisemann, M., & Richter, G. (2000). Temperament and character during the course of unipolar depression among inpatients. *European Archives of Psychiatry and Clinical Neuroscience*, 250, 40-47.

Risch, N., Burchard, E., Ziv, E., & Tang, H. (2002). Categorization of humans in biomedical research: Genes, race and disease. *Genome Biol*, 3(7), comment2007.

Risch, N., & Teng, J. (1998). The relative power of family-based and case-control designs for linkage disequilibrium studies of complex human diseases i. DNA pooling. *Genome Research*, 8(12), 1273-1288.

Risch, N. J. (2000). Searching for genetic determinants in the new millennium. *Nature*, 405, 847-856.

Risch, N. J., & Merikangas, K. R. (1996). The future of genetic studies of complex human diseases. *Science*, 273, 1516-1517.

Rohan, K. J., Roecklein, K. A., Nguyen, A. T., Johnson, L. G., & Handal, M. (2004). Comorbidity of seasonal affective disorder and the dsm-iv binge-eating criteria: More than just carbohydrate craving in winter. Paper presented at the Association for the Advancement of Behavior Therapy, New Orleans, LA.

Rohan, K. J., Sigmon, S. T., & Dorhofer, D. M. (2003). Cognitive-behavioral factors in seasonal affective disorder. *Journal of Consulting and Clinical Psychology*, 71(1), 22-30.

Rohan, K. J., Tierney Lindsey, K., Roecklein, K. A., & Lacy, T. J. (2004). Cognitive-behavioral therapy, light therapy, and their combination in treating seasonal affective disorder. *Journal of Affective Disorders*, 80, 273-283.

Rollag, M. D., Berson, D., & Provencio, I. (2003). Melanopsin, ganglion-cell photoreceptors, and mammalian photoentrainment. *Journal of Biological Rhythms*, 18(3), 227.

Rosen, L., Targum, S., Terman, M., Bryant, M., Hoffman, H., Kasper, S., et al. (1990). Prevalence of seasonal affective disorder at four latitudes. *Psychiatry Research*, 31(2), 131-144.

Rosenberg, N. A., Pritchard, J. K., Weber, J. L., Cann, H. M., Kidd, K. K., Zhivotovsky, L. A., et al. (2002). Genetic structure of human populations. *Science*, 298(5602), 2381-2385.

Rosenthal, N., Mazzanti, C., Barnett, R., Hardin, T., Turner, E., Lam, G., et al. (1998). Role of serotonin transporter promoter repeat length polymorphism (5-httlpr) in seasonality and seasonal affective disorder. *Molecular Psychiatry*, 3(2), 175-177.

Rosenthal, N. E. (2006). *Winter blues* (Revised ed.). New York: The Guilford Press.

Rosenthal, N. E., Bradt, G. H., & Wehr, T. A. (1984a). *Seasonal pattern assessment questionnaire*. Bethesda, MD: National Institute of Mental Health.

Rosenthal, N. E., Sack, D. A., Gillin, J. C., Lewy, A. J., Goodwin, F. K., Davenport, Y., et al. (1984b). Seasonal affective disorder. A description of the syndrome and preliminary findings with light therapy. *Archives of General Psychiatry*, 41(1), 72-80.

Ruby, N., Brennan, T., Xie, X., Cao, V., Franken, P., Heller, H., et al. (2002). Role of melanopsin in circadian responses to light. *Science*, 298(5601), 2211-2213.

Sasaki, T., Sakamoto, K., Akaho, R., Nakajima, T., & Takahashi, K. (1998). Familial transmission of seasonal changes in sleep and eating function in the general population. *Psychiatry Research*, 81(2), 211-217.

Schaid, D. J., & Rowland, C. (1998). Use of parents, sibs, and unrelated controls for detection of associations between genetic markers and disease. *American Journal of Human Genetics*, 63(5), 1492-1506.

Schinka, J. A., Busch, R. M., & Robichaux-Keene, N. (2004). A meta-analysis of the association between the serotonin transporter gene polymorphism (5-httlpr) and trait anxiety. *Molecular Psychiatry*, 9(2), 197-202.

Schork, N. J., Fallin, D., Thiel, B., Xu, X., Broeckel, U., Jacob, H. J., et al. (2001). The future of genetic case-control studies. *Advances in Genetics*, 42, 191-212.

Schork, N. J., Nath, S. K., Fallin, D., & Chakravarti, A. (2000). Linkage disequilibrium analysis of biallelic DNA markers, human quantitative trait loci, and threshold-defined case and control subjects. *American Journal of Human Genetics*, 67(5), 1208-1218.

Schwartz, P. J., Rosenthal, N. E., Kajimura, N., Han, L., Turner, E. H., Bender, C., et al. (2000). Ultradian oscillations in cranial thermoregulation and electroencephalographic slow-wave activity during sleep are abnormal in humans with annual winter depression. *Brain Research*, 866(1-2), 152-167.

Science, N. R. C. a. C. O. D. F. (1996). The evaluation of forensic DNA evidence. Washington, DC: National Academy Press.

Segal, D. L., Kabacoff, R. I., Hersen, M., Van Hasselt, V. B., & Ryan, C. F. (1995). Update on the reliability of diagnosis in older psychiatric outpatients using the structured clinical interview for dsm-iii-r. *Journal of Clinical Geropsychology*, 1, 313-321.

Sekaran, S., Foster, R. G., Lucas, R. J., & Hankins, M. W. (2003). Calcium imaging reveals a network of intrinsically light-sensitive inner-retinal neurons. *Current Biology*, 13(15), 1290-1298.

Serretti, A., Mandelli, L., Lorenzi, C., Landoni, S., Calati, R., Insacco, C., et al. (2005). Temperament and character in mood disorders: Influence of drd4, sertpr, tph and mao-a polymorphisms. *Neuropsychobiology*, 53(1), 9-16.

Shanahan, T. L., & Czeisler, C. A. (1991). Light exposure induces equivalent phase shifts of the endogenous circadian rhythms of circulating plasma melatonin and core body temperature in men. *Journal of Clinical Endocrinology and Metabolism*, 73(2), 227-235.

Shelton, R. C. (2000). Cellular mechanisms in the vulnerability to depression and response to antidepressants. *Psychiatric Clinics of North America*, 23(4), 713-729.

Sher, L. (2002). Relationships between seasonality and alcohol use: A genetic hypothesis. *Medical Hypotheses*, 59(1), 85-88.

Sher, L., Goldman, D., Ozaki, N., & Rosenthal, N. E. (1999). The role of genetic factors in the etiology of seasonal affective disorder and seasonality. *Journal of Affective Disorders*, 53(3), 203-210.

Sher, L., Greenberg, B. D., Murphy, D. L., Rosenthal, N. E., Sirota, L. A., & Hamer, D. H. (2000). Pleiotropy of the serotonin transporter gene for seasonality and neuroticism. *Psychiatric Genetics*, 10(3), 125-130.

Shriver, M. D., Kennedy, G. C., Parra, E. J., Lawson, H. A., Sonpar, V., Huang, J., et al. (2004). The genomic distribution of population substructure in four populations using 8,525 autosomal snps. *Hum Genomics*, 1(4), 274-286.

Shriver, M. D., Mei, R., Parra, E. J., Sonpar, V., Halder, I., Tishkoff, S. A., et al. (2005). Large-scale snp analysis reveals clustered and continuous patterns of human genetic variation. *Hum Genomics*, 2(2), 81-89.

Sinha, M., Larkin, E. K., Elston, R. C., & Redline, S. (2006). Self-reported race and genetic admixture. *New England Journal of Medicine*, 354(4), 421-422.

Skre, I., Onstad, S., Torgersen, S., & Kringlen, E. (1991). High interrater reliability for the structured clinical interview for dsm-iii-r axis i (scid-i). *Acta Psychiatrica Scandinavica*, 84(2), 167-173.

Smith, W. C., McDowell, J. H., Dugger, D. R., Miller, R., Arendt, A., Popp, M. P., et al. (1999). Identification of regions of arrestin that bind to rhodopsin. *Biochemistry*, 38(9), 2752-2761.

Sohn, C.-H., & Lam, R. W. (2005). Update on the biology of seasonal affective disorder. *CNS Spectrums*, 10(8), 635-646.

Sperling, W., Barocka, A., Kalb, R., Suss, S., & Katalinic, A. (1997). Influence of season on manifestation of schizophrenic subtypes. *Psychopathology*, 30(4), 200-207.

Spitzer, R. L., Endicott, J., & Robins, E. (1978). Research diagnostic criteria: Rationale and reliability. *Archives of General Psychiatry*, 35(6), 773-782.

Spitzer, R. L., Williams, J. B. W., Gibbon, M., & First, M. B. (1990). *Structured clinical interview for dsm-iii-r, patient edition/non-patient edition (scid-p/scid-np)*. Washington, D.C.: American Psychiatric Press, Inc.

Spoont, M. R., Depue, R. A., & Krauss, S. S. (1991). Dimensional measurement of seasonal variation in mood and behavior. *Psychiatry Research*, 39(3), 269-284.

Stephens, M., & Donnelly, P. (2003). A comparison of bayesian methods for haplotype reconstruction from population genotype data. *American Journal of Human Genetics*, 73(5), 1162-1169.

Stephens, M., Smith, N. J., & Donnelly, P. (2001). A new statistical method for haplotype reconstruction from population data. *American Journal of Human Genetics*, 68(4), 978-989.

Swedo, S. E., Pleeter, J. D., Richter, D. M., Hoffman, C. L., Allen, A. J., Hamburger, S. D., et al. (1995). Rates of seasonal affective disorder in children and adolescents. *American Journal of Psychiatry*, 152(7), 1016-1019.

Tanaka, O., & Kondo, H. (1994). Localization of mrnas for three novel members (beta 3, beta 4 and gamma 2) of phospholipase c family in mature rat brain. *Neuroscience Letters*, 182(1), 17-20.

Teicher, M. H., Glod, C. A., Magnus, E., Harper, D., Benson, G., Krueger, K., et al. (1997). Circadian rest-activity disturbances in seasonal affective disorder. *Archives of General Psychiatry*, 54(2), 124-130.

Terman, J., & Terman, M. (1999). Photopic and scotopic light detection in patients with seasonal affective disorder and control subjects. *Biological Psychiatry*, 46(12), 1642-1648.

Terman, J. S., Terman, M., Lo, E. S., & Cooper, T. B. (2001). Circadian time of morning light administration and therapeutic response in winter depression. *Archives of General Psychiatry*, 58(1), 69-75.

Terman, J. S., Terman, M., Schlager, D., Rafferty, B., Rosofsky, M., Link, M. J., et al. (1990). Efficacy of brief, intense light exposure for treatment of winter depression. *Psychopharmacology Bulletin*, 26(1), 3-11.

Terman, M. A., Terman, J. S., Quitkin, F. M., McGrath, P. J., Stewart, J. W., & Rafferty, B. (1989). Light therapy for seasonal affective disorder. A review of efficacy. *Neuropsychopharmacology*, 2(1), 1-22.

Terwilliger, J. D., & Weiss, K. M. (1998). Linkage disequilibrium mapping of complex disease: Fantasy or reality? *Current Opinion in Biotechnology*, 6, 578-594.

Thase, M., Jindal, R., & Howland, R. H. (2002). Biological aspects of depression. In I. H. Gotlib & C. L. Hammen (Eds.), Handbook of depression. New York: The Guilford Press.

Thase, M. E., Fasiczka, A. L., Berman, S. R., Simons, A. D., & Reynolds, C. F., 3rd. (1998). Electroencephalographic sleep profiles before and after cognitive behavior therapy of depression. *Archives of General Psychiatry*, 55(2), 138-144.

Thase, M. E., & Howland, R. H. (1995). Biological processes in depression: An updated review and integration. In E. E. Beckham & W. R. Leber (Eds.), Handbook of depression (2nd ed., pp. 213-279). New York: Guilford Press.

Thierry, N., Willeit, M., Praschak-Rieder, N., Zill, P., Hornik, K., Neumeister, A., et al. (2004). Serotonin transporter promoter gene polymorphic region (5-httlpr) and personality in female patients with seasonal affective disorder and in healthy controls. *European Neuropsychopharmacology*, 14(1), 53-58.

Thomas, D. C., & Witte, J. S. (2002). Point: Population stratification: A problem for case-control studies of candidate-gene associations? *Cancer Epidemiology, Biomarkers and Prevention*, 11(6), 505-512.

Thompson, C., & Isaacs, G. (1988). Seasonal affective disorder--a british sample. Symptomatology in relation to mode of referral and diagnostic subtype. *Journal of Affective Disorders*, 14(1), 1-11.

Thomson, G. (1995). Mapping disease genes: Family-based association studies. *American Journal of Human Genetics*, 57(2), 487-498.

Tiret, L., Poirier, O., Nicaud, V., Barbaux, S., Herrmann, S. M., Perret, C., et al. (2002). Heterogeneity of linkage disequilibrium in human genes has implications for association studies of common diseases. *Human Molecular Genetics*, 11(4), 419-429.

Twells, R. C., Mein, C. A., Phillips, M. S., Hess, J. F., Veijola, R., Gilbey, M., et al. (2003). Haplotype structure, ld blocks, and uneven recombination within the lrp5 gene. *Genome Research*, 13(5), 845-855.

Uno, H., Eisele, S., Sakai, A., Shelton, S., Baker, E., DeJesus, O., et al. (1994). Neurotoxicity of glucocorticoids in the primate brain. *Hormones and Behavior*, 28(4), 336-348.

Van Os, J., & Jones, P. B. (1999). Early risk factors and adult person--environment relationships in affective disorder. *Psychological Medicine*, 29(5), 1055-1067.

Wacholder, S., Chanock, S., Garcia-Closas, M., El Ghormli, L., & Rothman, N. (2004). Assessing the probability that a positive report is false: An approach for molecular epidemiology studies. *Journal of the National Cancer Institute*, 96(6), 434-442.

Wacholder, S., Rothman, N., & Caporaso, N. (2000). Population stratification in epidemiologic studies of common genetic variants and cancer: Quantification of bias. *Journal of the National Cancer Institute*, 92(14), 1151-1158.

Wang, S., & Zhao, H. (2003). Sample size needed to detect gene-gene interactions using association designs. *American Journal of Epidemiology*, 158(9), 899-914.

Wehr, T., Jacobsen, F., Sack, D., Arendt, J., Tamarkin, L., & Rosenthal, N. (1986). Phototherapy of seasonal affective disorder. Time of day and suppression of melatonin are not critical for antidepressant effects. *Archives of General Psychiatry*, 43(9), 870-875.

Wehr, T. A. (1999). Chronobiology. In B. J. Saddock & V. A. Saddock (Eds.), Comprehensive textbook of psychiatry (7th ed., Vol. 1, pp. 133-142): Lippincott Williams & Wilkins.

Wehr, T. A. (2001). Photoperiodism in humans and other primates: Evidence and implications. *Journal of Biological Rhythms*, 16(4), 348-364.

Wehr, T. A., Duncan, W. C., Jr., Sher, L., Aeschbach, D., Schwartz, P. J., Turner, E. H., et al. (2001). A circadian signal of change of season in patients with seasonal affective disorder. *Archives of General Psychiatry*, 58(12), 1108-1114.

Wehr, T. A., Schwartz, P. J., Turner, E. H., Feldman-Naim, S., Drake, C. L., & Rosenthal, N. E. (1995). Bimodal patterns of human melatonin secretion consistent with a two-oscillator model of regulation. *Neuroscience Letters*, 194(1-2), 105-108.

Weir, B. S. (1996). *Genetic data analysis ii: Methods for discrete population genetic data.* Sunderland, MA: Sinauer.

Weiss, J. M., & Kilts, C. D. (1998). Animal models of depression and schizophrenia. In A. F. Schatzberg & C. B. Nemeroff (Eds.), Textbook of psychopharmacology (2nd ed., pp. 89-131). Washington, DC: American Psychiatric Press.

Wienker, T., & Strom, T. (2003). Finetti. Retrieved August 1, 2005, 2005, from http://ihg.gsf.de/cgi-bin/hw/hwa1.pl

Williams, J. B. (1988). A structured interview guide for the hamilton depression rating scale. *Archives of General Psychiatry*, 45(8), 742-747.

Williams, J. B., Gibbon, M., First, M. B., Spitzer, R. L., Davies, M., Borus, J., et al. (1992a). The structured clinical interview for dsm-iii-r (scid). Ii. Multisite test-retest reliability. *Archives of General Psychiatry*, 49(8), 630-636.

Williams, J. B., Link, M. J., Rosenthal, N. E., Amira, L., & Terman, M. (1992b). *Structured interview guide for the hamilton depression rating scale - seasonal affective disorder version (sigh-sad).* New York: New York State Psychiatric Institute.

Williams, R. C., Long, J. C., Hanson, R. L., Sievers, M. L., & Knowler, W. C. (2000). Individual estimates of european genetic admixture associated with lower body-mass index, plasma glucose, and prevalence of type 2 diabetes in pima indians. *American Journal of Human Genetics*, 66, 527-538.

Willner, P. (1995). Dopaminergic mechanisms in depression and mania. In F. E. Bloom & D. J. Kupfer (Eds.), Psychopharmacology: The fourth generation of progress (pp. 921-931). New York: Raven Press.

Wirz-Justice, A., Bucheli, C., Graw, P., Kielholz, P., Fisch, H., & Woggon, B. (1986). Light treatment of seasonal affective disorder in switzerland. *Acta Psychiatrica Scandinavica*, 74(2), p193-204.

Wirz-Justice, A., Graw, P., Krauchi, K., Gisin, B., Jochum, A., Arendt, J., et al. (1993). Light therapy in seasonal affective disorder is independent of time of day or circadian phase. *Archives of General Psychiatry*, 50(12), 929-937.

Wirz-Justice, A., Krauchi, K., Brunner, D., Graw, P., Haug, H. J., Leonhardt, G., et al. (1995). Circadian rhythms and sleep regulation in seasonal affective disorder. *Acta Neuropsychiatrica*, 7, 41-43.

Xu, K., Lichtermann, D., Lipsky, R. H., Franke, P., Liu, X., Hu, Y., et al. (2004). Association of specific haplotypes of d2 dopamine receptor gene with vulnerability to heroin dependence in 2 distinct populations. *Archives of General Psychiatry*, 61(6), 597-606.

Yannielli, P., & Harrington, M. E. (2004). Let there be "More" Light: Enhancement of light actions on the circadian system through non-photic pathways. *Progress in Neurobiology*, 74(1), 59-76.

Yoney, T. H., Pigott, T. A., L'Heureux, F., & Rosenthal, N. E. (1991). Seasonal variation in obsessive-compulsive disorder: Preliminary experience with light treatment. *American Journal of Psychiatry*, 148(12), 1727-1729.

Young, M. A. (1999). Integrating psychological and physiological mechanisms of sad: The dual-vlunerability model. *Biological Rhythms Bulletin*, 1(3), 4-6.

Young, M. A., Blodgett, C., & Reardon, A. (2003). Measuring seasonality: Psychometric properties of the seasonal pattern assessment questionnaire and the inventory for seasonal variation. *Psychiatry Research*, 117(1), 75-83.

Young, M. A., Meaden, P. M., Fogg, L. F., Cherin, E. A., & Eastman, C. I. (1997). Which environmental variables are related to the onset of seasonal affective disorder? *Journal of Abnormal Psychology*, 106(4), 554-562.

Young, M. A., Watel, L. G., Lahmeyer, H. W., & Eastman, C. I. (1991). The temporal onset of individual symptoms in winter depression: Differentiating underlying mechanisms. *Journal of Affective Disorders*, 22(4), 191-197.

Zanarini, M. C., & Frankenburg, F. R. (2001). Attainment and maintenance of reliability of axis i and ii disorders over the course of a longitudinal study. *Comprehensive Psychiatry*, 42(5), 369-374.

Zheng, W., & Cole, P. A. (2002). Serotonin n-acetyltransferase: Mechanism and inhibition. *Current Medicinal Chemistry*, 9(12), 1187-1199.

## APPENDIX A

## STRUCTURED INTERVIEW FOR THE HAMILTON DEPRESSION RATING SCALE SEASONAL AFFECTIVE DISORDER VERSION (SIGH-SAD)

OVERVIEW: I'd like to ask you some questions about the past week, since last (DAY OF WEEK). How have you been feeling since then?

H1. What's your mood been like this past week (compared to when you feel OK)?

Have you been feeling down or depressed?

Sad? Hopeless? Helpless? Worthless?

In the last week, how often have you felt (OWN EQUIVALENT)? Every day? All day?

Have you been crying at all?

DEPRESSED MOOD (sadness, hopeless, helpless, worthless):

0 = absent
1 = indicated only on questioning
2 = spontaneously reported verbally
3 = communicated non-verbally, i.e. facial expression, posture, voice tendency to weep
4 = VIRTUALLY ONLY; this in spontaneous verbal and non-verbal communication

IF SCORED 1-4 ABOVE, ASK: How long have you been feeling this way?

H2. IF OUTPATIENT: Have you been working this week (in or out of the home)?
IF NOT: Why not?

IF WORKING: Have you been able to get as much (work) done as you usually do (when you're feeling OK)?

How have you been spending your time past week (when not at work)?

Have you felt interested in doing (THOSE THINGS), or do you feel you have to push yourself to do them?

Have you stopped doing anything you used to do? IF YES: Why?

Is there anything you look forward to?

WORK AND ACTIVITIES:

0 = no difficulty
1 = thoughts and feelings of incapacity, fatigue or weakness related to activities, work or hobbies
2 = loss of interest in activity, hobbies or work – by direct report of the patient or indirect in listlessness, indecision this and vacillation (feels he has to push self to do work or activities)
3 = decrease in actual time spent in activities or decrease in productivity. In hospital, patient spends less than 3 hours/day in activities (hospital job or hobbies) exclusive of ward chores

4 = stopped working because of present illness.In hospital, no activities except ward chores, or fails to perform ward chores unassisted

A1. In the last week, have you been as social as when you feel well?
IF NO: Tell me which fits you best.
(READ DOWN ANCHOR DESCRIPTIONS AND RATE ACCORDINGLY.)

*SOCIAL WITHDRAWAL:

0 = interacts with other people as usual
1 = less interested in socializing with others but continues to do so
2 = interacting less with other people in social (optional) situations
3 = interacting less with other people in work or family situations (i.e., where it is necessary)
4 = marked withdrawal from others in family or work situations

H3. This week, how has your interest in sex been? (I'm not asking about actual sexual activity, but about your interest in sex – how much you think about it.)

Has there been any change in your interest in sex (from when you were not depressed)?

Is it something you've thought much about?
IF NO: Is that unusual for you compared to when you feel well? (Is it a little less or a lot less?)

GENITAL SYMPTOMS (such as loss of libido, menstrual disturbances):

0 = absent
1 = mild
2 = severe

H4. How has your appetite been this past week? (What about compared to your usual appetite?)

Have you had to force yourself to eat?

Have other people had to urge you to eat? (Have you skipped meals?)

Have you had any stomach or intestinal problems? (Have you needed to take anything for that?)

SOMATIC SYMPTOMS GASTROINTESTINAL:

0 = none
1 = loss of appetite but eating without encouragement
2 = difficulty eating without urging: requests or requires laxatives or medication for G.I. symptoms

H5. Have you lost any weight since you started feeling depressed or down? IF YES: Did you lose any weight this last week? (Was it because of feeling depressed?) How much did you lose?

IF NOT SURE: Do you think your clothes are any looser on you?

LOSS OF WEIGHT (Rate either A or B):

A. When rating by history:
0 = no weight loss
1 = probable weight loss due to current depression
2 = definite (according to patient) weight loss due to depression
3 = not assessed

B. When actual weight changes are measured:
0 = less than 1 pound loss in week
1 = greater than 1 pound loss in week
2 = greater than 2 pounds loss in week
3 = not assessed

A2. Have you gained any weight in the last week? IF YES: Was it because of feeling depressed or down? How much did you gain?

*WEIGHT GAIN:

0 = no weight gain
1 = probable weight gain due to current depression
2 = definite (according to patient) weight gain due to depression

A3. In the past week, has your appetite been greater than when you feel well or OK? IF YES: Do you want to eat a little more, somewhat more, or much

*APPETITE INCREASE:

0 = no increase in appetite
1 = wants to eat a little more than usual more than when you feel well or OK?
2 = wants to eat somewhat more than normal
3 = wants to eat much more than usual

A4. In the past week, have you actually been eating more than when you feel well or OK? IF YES: A little more, somewhat more, or much more than when you feel well or OK?

*INCREASED EATING

0 = is not eating more than usual
1 = is eating a little more than usual
2 = is eating somewhat more than usual
3 = is eating much more than normal

A5. In the last week, have you been craving or eating more starches or sugars? IF YES: Have you been eating or craving starches or sugars more than when you feel well or OK, much more, or has it been irresistible?

*CARBOHYDRATE CRAVING OR EATING (in relation to total amount of food desired or eaten)
0 = no change in food preference or consumption
1 = craving or eating more carbohydrates (starches or sugars) than before
2 = craving or eating much more carbohydrates than before
3 = irresistible craving or eating of sweets or starches

Has it been mainly starches or mainly sweets? Which specific foods have you been craving?
LIST:

CIRCLE ONE OR BOTH: Mainly starches | Mainly sweets | Both

Have you actually been eating more starches or sweets, or just craving them?

CIRCLE ONE OR BOTH: Craving | Eating | Both

Has the (CRAVING OR EATING) occurred at any particular time of day? (_____o'clock)

USUAL TIME OF CRAVING OR EATING:

0 = it comes and goes at various times
1 = usually morning
2 = usually afternoon or evening
3 = virtually all the time

RATER NOTE: IF BOTH CRAVING AND EATING, RATE TIME OF EATING. DO NOT COUNT ABOVE SCORE IN TOTALS.

H6. I'd like to ask you now about your sleeping during the past week.

Have you had any trouble falling asleep at the beginning of the night? (Right after you go to bed, how long has it been taking you to fall asleep?)

How many nights this week have you had trouble falling asleep?

INSOMNIA EARLY (INITIAL INSOMNIA):

0 = no difficulty falling asleep
1 = complains of occasional difficulty falling asleep – i.e., more than ½ hour
2 = complains of nightly difficulty falling asleep

H7. During the past week, have you been waking up in the middle of the night?
IF YES: Do you get out of bed? What do you do? (Only go to the bathroom?)

When you get back in bed, are you able able to fall right back asleep?
Have you felt your sleeping has been restless or disturbed some nights?

INSOMNIA MIDDLE:

0 = no difficulty
1 = complains of being restless and disturbed during the night
2 = waking during the night – any getting out of bed (except to void)

H8. What time have you been waking up in the morning for the last time, this past week?

IF EARLY: Is that with an alarm clock, or do you just wake up yourself?

What time do you usually wake up (that is, when you feel well)?

INSOMNIA LATE (TERMINAL INSOMNIA):
0 = no difficulty
1 = waking in early hours of morning but goes back to sleep
2 = unable to fall asleep again if gets out of bed

A6. Have you been sleeping more than usual this past month?
IF YES: How much more?
IF NO: What about weekends?
use 8 hours):

(What time have you been falling asleep? Have you been taking naps? That means you've been sleeping about ___ hours a day altogether? How much time do you usually sleep when you feel well?)

*HYPERSOMNIA (Compare sleep Length to euthymic and NOT to euthymic and NOT to hypomanic sleep length. (If this cannot be established,

0 = no increase in sleep length
1 = at least 1 hour increase in sleep length
2 = 2-hour increase
3 = 3-hour increase
4 = 4-hour increase

Sleep length used (circle one):

euthymic (___ hrs) 8-hour

H9. How has your energy been this past week?

IF LOW ENERGY: Have you felt tired? (How much of the time? How bad has it been?)

This week, have you had any aches or pains? (What about backaches, headaches, or muscle aches?

Have you felt any heaviness in your limbs, back or head?

SOMATIC SYMPTOMS GENERAL:

0 = none
1 = heaviness in limbs, back or head. Backaches, headaches, muscle aches. Loss of energy and fatigability.
2 = any clear-cut symptom

A7. IF ACKNOWLEDGED FEELING TIRED ON PREVIOUS ITEM: How much of the time have you felt tired? (Every day? How much of each day?)

Very tired, or just a little?

*FATIGABILITY (or low energy, or feelings of being heavy, leaden, weighed down);

0 = does not feel more fatigued than usual
1 = feels more fatigued than usual but this has not impaired function significantly; less frequent than in (2)
2 = more fatigued than usual; at least one hour a day; at least three days a week
3 = fatigued much of the time most days
4 = fatigued almost all the time

H10. Have you been putting yourself down, this past week, feeling you've done things wrong, or let others down?
If Yes: What have your thoughts been?

Have you been feeling guilty about anything that you've done or not done? What about things that happened a long time ago?

Have you thought that you've brought (THIS DEPRESSION) on yourself in same way?

Do you feel your being sick is a punishment?

FEELINGS OF GUILT:

0 = absent
1 = self-reproach, feels he/she has let people down
2 = ideas of guilt or rumination over past errors or sinful deeds
3 = present illness is a punishment: delusions of guilt
4 = hears accusatory or denunciatory voices and/or experiences threatening visual hallucinations

H11. This past week, have you had any thoughts that life is not worth living? IF YES: What about thinking you'd be better off dead? Have you had thoughts of hurting or killing yourself?

IF YES: What have you thought about? Have you actually done anything to hurt yourself?

SUICIDE:

0 = absent
1 = feels life is not worth living
2 = wishes he were dead or any thoughts of possible death to self
3 = suicidal ideas or gesture
4 = attempts at suicide

H12. Have you been feeling especially tense or irritable this past week? IF YES: Is this more than when you are not depressed or down?

Have you been unusually argumentative or impatient?

Have you been worrying a lot about little things, things you don't ordinarily worry about? IF YES: Like what, for example?

ANXIETY PSYCHIC:

0 = no difficulty
1 = subjective tension and irritability
2 = worrying about minor matters
3 = apprehensive attitude apparent in face or speech
4 = fears expressed without questioning

H13. In this past week, have you had any of the following physical symptoms? (READ LIST, PAUSING AFTER EACH SX FOR REPLY. CIRCLE POSITIVE SXS.)

Have you had these only while you've been feeling depressed or down? IF YES: How much have these things been bothering you this past week? (How bad have they gotten? How much of the time, or how often, have you had them?)

Do you have any physical illness or are you taking any medication that could be causing these symptoms? (IF YES, RECORD PHYSICAL ILLNESS OR MEDICATION, BUT RATE SYMPTOMS ANYWAY:_____)

ANXIETY SOMATIC (physiologic concomitants of anxiety, such as
GI – dry mouth, indigestion, gas diarrhea, stomach cramps, belching
C-V- heart palpitations, headaches
Resp – hyperventilating, sighing
Having to urinate frequently
Sweating):

0 = absent
1 = mild
2 = moderate
3 = severe
4 = incapacitating

H14. In the last week, how much have your thoughts been focused on your physical health or how your body is working (compared to your normal thinking)? (Have you worried a lot about being or becoming physically ill? Have you really been preoccupied with this?)

Do you complain much about how you feel physically?

Have you found yourself asking for help with things you could really do yourself? IF YES: Like what, for example? How often has that happened?

HYPOCHONDRIASIS:

0 = not present
1 = self-absorption (bodily)
2 = preoccupation with health
3 = frequent complaints, requests for help, etc.
4 = hypochondriacal delusions

H15. RATING BASED ON OBSERVATION DURING INTERVIEW.

INSIGHT:

0 = acknowledges being depressed and ill OR not currently depressed
1 = acknowledges illness but attributes cause to bad food, overwork, virus, need for rest, etc.
2 = denies being ill at all

H16. RATING BASED ON OBSERVATION DURING INTERVIEW
concentrate; decreased motor activity): IF TELEPHONE INTERVIEW: Do you feel that your speech or physical movements are sluggish? Has anyone actually commented on this?

RETARDATION (slowness of thought and speech; impaired ability to

0 = normal speech and thought
1 = slight retardation at interview
2 = obvious retardation at interview
3 = interview difficult
4 = complete stupor

H17. RATING BASED ON OBSERVATION INTERVIEW.

IF TELEPHONE INTERVIEW: As we talk, are you fidgeting at all, or having trouble sitting still? For instance, are you doing anything like playing with your hands or your hair, or tapping your foot? Do others notice that you are restless?

AGITATION:

0 = none
1 = fidgetiness
2 = playing with hands, hair, etc.
3 = moving about, can't sit still
4 = hand- wringing, nail biting, hair-pulling, biting of lips

17-ITEM TOTAL SCORE HAMILTON DEPRESSION _____ _____

Over the past week, in the first few hours after waking up have you been feeling better or worse or no different from before you go to sleep?

DIURNAL VARIATION TYPE A:

A. Note whether symptoms are worse after awakening or before sleeping. If NO diurnal variation, mark none:
0 = no variation OR not currently depressed
1 = worse after awakening
2 = worse before going to sleep

RATER NOTE: DO NOT COUNT ABOVE SCORE IN SCALE TOTALS.

H18. IF VARIATION: How much worse do you feel in the (MORNING OR EVENING)? IF UNSURE: A little bit worse or a lot worse?

B. When present, mark the severity of the variation:
0 = none
1 = mild
2 = severe

A8. This week, have you regularly had a slump in your mood or energy in the afternoon or evening?

IF YES: Is it mostly in your mood or your energy? Does it occur every day? At what time has the slump usually begun? (_____o'clock). When has it ended? Has that been at least an hour before you go to sleep? How big a slump do you have - would you say it's generally mild, moderate, or severe?
HOUR OF RECOVERED MOOD OR

*DIURNAL VARIATION TYPE B:

0 = no
1 = yes, of mild intensity
2 = yes, of moderate intensity
3 = yes, of severe intensity

| CIRCLE ONE OR BOTH: | Mood Slump | Energy Slump |
|---|---|---|

NOTE: RATE ONLY SLUMPS THAT ARE FOLLOWED BY AT LEAST AN ENERGY BEFORE SLEEP.

H19. In the past week, have you ever suddenly had the sensation that everything is unreal, or you're in a dream, or cut off people in some strange way?

IF YES: Tell me about it. How bad has that been? How often this week has that happened?

DEPERSONALIZATION AND DEREALIZATION
(such as feelings of unreality and from other nihilistic ideas):

0 = absent
1 = mild
2 = moderate
3 = severe
4 = incapacitating

H20. This past week, have you thought that anyone was trying to give you a hard time or hurt you?

What about talking about you behind your back?

IF YES: Tell me about that.

PARANOID SYMPTOMS:

0 = none
1 = suspicious
2 = ideas of reference
3 = delusions of reference and persecution

H21. In the past week, have there been things you've had to do over and over again, like checking the locks on the doors several times, or washing your hands? IF YES: Can you give me an example?

Have you had any thoughts that don't make any sense to you, but that keep running over and over in your mind? IF YES: Can you give me an example?

OBSESSIONAL AND COMPULSIVE SYMPTOMS:

0 = absent
1 = mild
2 = severe

21-ITEM TOTAL SCORE HAMILTON DEPRESSION (without starred items): _____ _____

TOTAL 8-ITEM ATYPICAL SCORE (starred items only): _____ _____

TOTAL 29-ITEM SIGH-SAD SCORE _____ _____

ATYPICAL BALANCE SCORE (total 8-item atypical score divided by total 29-item SIGH-SAD score, multiplied by 100): ____ ____ . ____

NOTE: If patient is not depressed and score is derived primarily from symptoms of hypomania (e.g., items H4, H5, H6, H7, H8, H12, H17), administer HIGH-SAD and report both scores.

## APPENDIX B

## SEASONAL PATTERN ASSESSMENT QUESTIONNAIRE

**1. Name** _____ **2. Age** _____

**3. Place of birth - City / Province (State) / Country** _____

**4. Today's date** _____ _____ _____
Month Day Year

**5. Current weight (in lbs.)** _____

**6. Years of education**

| | |
|---|---|
| Less than four years of high school | 1 |
| High school only | 2 |
| 1-3 years post high school | 3 |
| 4 or more years post high school | 4 |

**7. Sex -** Male 1 Female 2

**8. Marital Status -**

| | |
|---|---|
| Single | 1 |
| Married | 2 |
| Sep./Divorced | 3 |
| Widowed | 4 |

**9. Occupation** _____

**10. How many years have you lived in this climatic area?** _____

**INSTRUCTIONS**

* Please circle the number beside your choice.

Example:
Sex Male 1 Female 2

**The purpose of this form is to find out how your mood and behaviour change over time. Please fill in all the relevant circles. Note: We are interested in your experience; not others you may have observed.**

**11. To what degree do the following change with the seasons?**

| | No Change | Slight Change | Moderate Change | Marked Change | Extremely Marked Change |
|---|---|---|---|---|---|
| A. Sleep length | 0 | 1 | 2 | 3 | 4 |
| B. Social activity | 0 | 1 | 2 | 3 | 4 |
| C. Mood (overall feeling of well being) | 0 | 1 | 2 | 3 | 4 |
| D. Weight | 0 | 1 | 2 | 3 | 4 |
| E. Appetite | 0 | 1 | 2 | 3 | 4 |
| F. Energy level | 0 | 1 | 2 | 3 | 4 |

**12. In the following questions, fill in circles for all applicable months. This may be a single month O, a cluster of months, e.g. O O O , or any other grouping.**

**At what time of year do you....**

| | Jan | Feb | Mar | Apr | May | Jun | Jul | Aug | Sep | Oct | Nov | Dec | OR | No particular month(s) stand out as extreme on a regular basis |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A. Feel best | O | O | O | O | O | O | O | O | O | O | O | O | | O |
| B. Gain most weight | O | O | O | O | O | O | O | O | O | O | O | O | | O |
| C. Socialize most | O | O | O | O | O | O | O | O | O | O | O | O | | O |
| D. Sleep least | O | O | O | O | O | O | O | O | O | O | O | O | | O |
| E. Eat most | O | O | O | O | O | O | O | O | O | O | O | O | **OR** | O |
| F. Lose most weight | O | O | O | O | O | O | O | O | O | O | O | O | | O |
| G. Socialize least | O | O | O | O | O | O | O | O | O | O | O | O | | O |
| H. Feel worst | O | O | O | O | O | O | O | O | O | O | O | O | | O |
| I. Eat least | O | O | O | O | O | O | O | O | O | O | O | O | | O |
| J. Sleep most | O | O | O | O | O | O | O | O | O | O | O | O | | O |

**14. How much does your weight fluctuate during the course of the year?**

| | | | |
|---|---|---|---|
| 0-3 lbs | 1 | 12-15 lbs | 4 |
| 4-7 lbs | 2 | 16-20 lbs | 5 |
| 8-11 lbs | 3 | Over 20 lbs | 6 |

**15. Approximately how many hours of each 24-hour day do you sleep during each season? (include naps)**

| | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Winter | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | Over18 |
| Spring | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | Over18 |
| Summer | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | Over18 |
| Fall | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | Over18 |

**16. Do you notice a change in food preference during the different seasons?**

No 1 Yes 2 If yes, please specify :

**17. If you experience changes with the seasons, do you feel that these are a problems for you?**

| | | | | |
|---|---|---|---|---|
| No 1 | Yes 2 | If yes, is this problem - | mild | 1 |
| | | | moderate | 2 |
| | | | marked | 3 |
| | | | severe | 4 |
| | | | disabling | 5 |

**Thank you for completing this questionnaire.**

* Raymond W. Lam 1998 (modified from Rosenthal, Bradt and Wehr 1987).